(FILE 'HOME' ENTERED AT 16:03:34 ON 05 OCT 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS, CANCERLIT, SCISEARCH, TOXLINE'
ENTERED AT 16:04:01 ON 05 OCT 2001

L1 37918 S NEU OR ERBB2 OR ERBB-2 OR CERBB-2 OR C-ERBB2 OR CERBB2 OR C-E
L2 4336 S P100
L3 9 S L1 (30A) L2
L4 4 DUP REM L3 (5 DUPLICATES REMOVED)
L5 35141 S EXTRACELLULAR DOMAIN OR ECD
L6 1116 S L1 (30A) L5
L7 813 S L1 (3A) L5
L8 14 S ISOLATE# (30A) L6
L9 5 DUP REM L8 (9 DUPLICATES REMOVED)
L10 671365 S DALTONS OR DA OR KDA
L11 765 S L1 (30A) L10
L12 515 S L1 (10A) L10
L13 57 S L10 (10A) ((BREAST)(10A)(ANTIGEN OR AG))
L14 15 DUP REM L13 (42 DUPLICATES REMOVED)

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
1	BRS	L1	3409	neu or erbb-2 or erbb2 or cerbb2 or c-erbb2 or cerbb-2 or c-erbb-2 or p185 or her-2 or her2	USPAT	2001/10/05 15:51			0
2	BRS	L2	622	p100	USPAT	2001/10/05 14:21			0
3	BRS	L3	2	1 same 2	USPAT	2001/10/05 14:22			0
4	BRS	L4	3868	ecd or (extracellular adj1 domain)	USPAT	2001/10/05 15:52			0
5	BRS	L5	151	1 same 4	USPAT	2001/10/05 14:22			0
6	BRS	L6	13	1 same 4 .clm.	USPAT	2001/10/05 14:26			0
7	BRS	L7	124	1 near40 4	USPAT	2001/10/05 14:27			0
8	BRS	L9	119	1 near10 4	USPAT	2001/10/05 15:42			0
9	BRS	L10	38876	daltons or Da or kda	USPAT	2001/10/05 15:56			0
10	BRS	L11	112	1 same 10	USPAT	2001/10/05 15:42			0
11	BRS	L12	52	1 near4 10	USPAT	2001/10/05 15:47			0
12	BRS	L13	8377	breast near3 (cancer or tumor$1 or tumour$1 or malignan$4 or neoplas$3)	USPAT	2001/10/05 15:49			0
13	BRS	L14	20	10 near4 13	USPAT	2001/10/05 15:50			0

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
14	BRS	L15	209	p100	US-PGPUB; EPO; JPO; DERWENT	2001/10/05 15:51			0
15	BRS	L16	1213	neu or erbb-2 or erbb2 or cerbb2 or c-erbb2 or cerbb-2 or c-erbb-2 or p185 or her-2 or her2	US-PGPUB; EPO; JPO; DERWENT	2001/10/05 15:51			0
16	BRS	L17	5	15 same 16	US-PGPUB; EPO; JPO; DERWENT	2001/10/05 15:51			0
17	BRS	L18	1395	ecd or (extracellular adj1 domain)	US-PGPUB; EPO; JPO; DERWENT	2001/10/05 15:52			0
18	BRS	L19	25	16 same 18	US-PGPUB; EPO; JPO; DERWENT	2001/10/05 15:53			0

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
19	BRS	L20	18175	daltons or Da or kda	US-PGPUB; EPO; JPO; DERWENT	2001/10/05 15:57			0
20	BRS	L21	14	16 same 20	US-PGPUB; EPO; JPO; DERWENT	2001/10/05 15:57			0

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
1	BRS	L1	2859	neu	USPAT	2001/10/06 12:46			0
2	BRS	L2	3868	ecd or (extracellular adj1 domain)	USPAT	2001/10/06 12:47			0
3	BRS	L3	44	1 same 2	USPAT	2001/10/06 12:54			0
4	BRS	L4	425	carney.in.	USPAT	2001/10/06 12:55			0
5	BRS	L5	3	1 and 4	USPAT	2001/10/06 12:55			0

(FILE 'HOME' ENTERED AT 16:08:46 ON 06 OCT 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS, CANCERLIT, SCISEARCH, TOXLINE'
ENTERED AT 16:09:08 ON 06 OCT 2001

L1 2375 S OD-3 OR NB-3 OR TA-1 OR (HB (W)(10204 OR 10205 OR 10206))
L2 1972239 S ANTIBODY OR AB OR MAB
L3 133 S L1 (P) L2
L4 45 DUP REM L3 (88 DUPLICATES REMOVED)

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
1	BRS	L1	154	OD-3 OR NB-3 OR TA-1 OR (HB adj1("10204" OR "10205" OR "10206"))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/10/06 16:18			0
2	BRS	L2	270951	ANTIBOD$3 OR AB OR MAB	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/10/06 16:18			0
3	BRS	L3	30	1 same 2	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/10/06 16:18			0

13042319 BIOSIS Number: 99042319
Humoral and cellular responses raised against the human HER2 oncoprotein are cross-reactive with the homologous product of the neu proto-oncogene, but do not protect rats against B104 tumors expressing mutated neu
Taylor P; Gerder M; Moros Z; Feldmann M
IVIC, BAMCO.CCS.199.00., PO Box 025322, Miami, FL 33102-5322, USA
Cancer Immunology Immunotherapy 42 (3). 1996. 179-184.
Full Journal Title: Cancer Immunology Immunotherapy
ISSN: 0340-7004
Language: ENGLISH
Print Number: Biological Abstracts Vol. 102 Iss. 002 Ref. 024492
The neu proto-oncogene encodes a plasma membrane protein belonging to the epidermal growth factor receptor family. The cell line B 104, derived from a BDIX rat neuroblastoma, carries a point mutation in neu, and forms a tumor when injected into these rats. The human homologue of the neu oncogene (here called HER2) is overexpressed in certain types of cancer. Rats were immunized with HER2 protein (HER2) toinvestigate a possible cross-reaction between the homologous proteins which could protect them against subsequent inoculation with B 104. Specific antibody in the serum was measured by cell-based enzyme-linked immunosorbent assay and fluorescence immunocytochemistry, and delayed-type hypersensitivity by an ear assay. Sera from animals immunized with the HER2 extracellular domain (HER2-ECD) reacted with both HER2- and neu-expressing cells. In the ear assay, a significant cellular response to both HER2-ECD (P lt 0.05) and neu protein (P lt 0.001) was observed in HER2-ECD-immunized rats. However, the growth of B 104 tumors in rats was not affected by preimmunization with HER2-ECD. The results indicate that an autoreactive immune response to neu was induced by immunization with HER2-ECD, but was too weak to affect the growth of the neu-bearing tumor.

9/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

12229853 BIOSIS Number: 98829853
Peptide-based, but not whole protein, vaccines elicit immunity to HER-2-neu, an oncogenic self-protein
Disis M L; Gralow J R; Bernard H; Hand S L; Rubin W D; Cheever M A
Div. Oncol., Box 356527, 1959 N.E. Pacific St., Health Sci. Build., BB1321, Seattle, WA 98195-6527, USA
Journal of Immunology 156 (9). 1996. 3151-3158.
Full Journal Title: Journal of Immunology
ISSN: 0022-1767
Language: ENGLISH
Print Number: Biological Abstracts Vol. 101 Iss. 012 Ref. 180275
HER-2/neu, an overexpressed oncogenic protein, has been proposed as a human cancer vaccine target. HER-2/neu is a "self" protein, however, and methods of vaccine strategies that would be effective in immunizing patients to a "self" tumor Ag have not been established. Many of the tumor Ags defined in humans are nonmutated self proteins, e.g., MAGE, and overcoming tolerance may be key in the generation of effective anti-tumor immunity. One theory states that tolerance to self proteins is directed only to dominant epitopes of proteins and not to every portion of the protein. Accordingly, tolerance can be circumvented by immunization to peptide fragments, but not whole protein. The studies outlined here demonstrate rat neu-specific immunity could be elicited in rats by

rat neu protein is 89% homologous to human HER-2/neu protein

vaccination with immunogenic rat neu peptides, but not by immunization with the intact protein. A rat model was used since rat neu protein is 89% homologous to human HER-2/neu protein and has a similar tissue distribution and level of expression. Rats were immunized with groups of peptides derived from the amino acid sequence of the intracellular domain or extracellular domain of rat neu protein and both groups developed CD4+ T cell immunity and Ab immunity to rat neu peptides and protein. Animals immunized in a similar fashion with intact purified rat neu protein did not develop Ab or T cell immunity to rat neu. Furthermore, rats that developed neu-specific immunity showed no histopathologic evidence of autoimmunity directed against organs expressing basal levels of rat neu protein. These studies suggest an immunization strategy that might be effective in human cancer vaccines targeting self tumor Ag.

9/7/3 (Item 3 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

8624389 BIOSIS Number: 92089389
ONCOGENIC FORMS OF THE NEU-HER2 TYROSINE KINASE ARE PERMANENTLY COUPLED TO PHOSPHOLIPASE C-GAMMA
PELES E; BEN LEVY R; OR E; ULLRICH A; YARDEN Y
DEP. CHEMICAL IMMUNOL., WEIZMANN INST. SCI., REHOVOT 76100, ISRAEL.
EMBO (EUR MOL BIOL ORGAN) J 10 (8). 1991. 2077-2086. CODEN: EMJOD
Full Journal Title: EMBO (European Molecular Biology Organization) Journal
Language: ENGLISH
The neu/HER2 proto-oncogene encodes a transmembrane tyrosine kinase homologous to receptors for polypeptide growth factors. The oncogenic potential of the presumed receptor is released through multiple genetic mechanisms including specific point mutation, truncation at the extracellular domain and overexpression of the proto-oncogene. Here we show that all these modes of oncogenic activation result in constitutively phosphorylated neu protein and an increase in tyrosine phosphorylation of a phosphatidylinositol-specific phospholipase (PLC.gamma.). The examined transforming neu/HER2 proteins, unlike the normal gene product, also co-immunoprecipitated with PLC.gamma. molecules. A kinase-defective mutant of a transforming neu failed to mediate both tyrosine phosphorylation and association with PLC.gamma., suggesting direct interaction of the neu kinase with PLC.gamma.. This possibility was examined by employing a chimeric protein composed of the extracellular ligand-binding domain of the epidermal growth factor receptor and the neu cytoplasmic portion. The chimeric receptor mediated rapid ligand-dependent modification of PLC.gamma. on tyrosine residues. It also physically associated, in a ligand-dependent manner, with the phosphoinositidase. Based on the presented results we suggest that the mechanism of cellular transformation by the neu/HER2 receptor involves tyrosine phosphorylation and activation of PLC.gamma..

9/7/4 (Item 4 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

7032047 BIOSIS Number: 87092568
A CHIMERIC EGF-R-NEU PROTO-ONCOGENE ALLOWS EGF TO REGULATE NEU TYROSINE KINASE AND CELL TRANSFORMATION

LEHVASLAIHO H; LEHTOLA L; SISTONEN L; ALITALO K
DEP. VIROL. PATHOL., UNIV. HELSINKI, HAARTMANINKATU 3, SF-00290 HELSINKI, FINL.
EMBO (EUR MOL BIOL ORGAN) J 8 (1). 1989. 159-166. CODEN: EMJOD
Full Journal Title: EMBO (European Molecular Biology Organization) Journal
Language: ENGLISH

The neu oncogene, characterized by Weinberg and colleagues, is a transforming gene found in ethylnitrosourea-induced rat neuro/glioblastomas; its human protooncogene homologue has been termed erbB2 or HER2 because of its close homology with the epidermal growth factor receptor (EGF-R) gene (c-erbB1). Expression of the rat neu oncogene is sufficient for transformation of mouse NIH 3T3 fibroblasts in culture and for the development of mammary carcinomas in transgenic mice, but the neu proto-oncogene has not been associated with cell transformation. We constructed a vector for expression of a chimeric cDNA and hybrid protein consisting of the EGF-R extracellular, transmembrane and protein kinase C-substrate domains linked to the intracellular tyrosine kinase and carboxyl terminal domain of the rat neu cDNA. Upon transfection with the construct, NIH 3T3 cells gave rise to EGF-R antigen-positive cell clones with varying amounts of specific EGF binding. Immunofluorescence and immunoprecipitation using neu- and EGF-receptor specific antibodies demonstrated a correctly oriented and positioned chimeric EGF-R-neu protein of the expected apparent mol. wt on the surface of these cells. EGF or TGF.alpha. induced tyrosine phosphorylation of the chimeric receptor protein, stimulated DNA synthesis of EGF-R-neu expressing cells and led to a transformed cell morphology and growth in soft agar. In contrast, the neur proto-oncogene did not show kinase activity or transforming properties when expressed at similar levels in NIH 3T3 cells. These results suggest that the neu proto-oncogene possesses mitogenic and transforming properties only in the presence of a ligand which stimulates its tyrosine kinase activity and provides the first model for studies of the function of the neu tyrosine kinase.

9/7/5 (Item 5 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

5751244 BIOSIS Number: 83013551
ALTERNATIVE 5' EXONS AND TISSUE-SPECIFIC EXPRESSION OF THE DROSOPHILA EPIDERMAL GROWTH FACTOR RECEPTOR HOMOLOG TRANSCRIPTS
SCHEJTER E D; SEGAL D; GLAZER L; SHILO B-Z
DEPARTMENT OF VIROLOGY, WEIZMANN INSTITUTE OF SCIENCE, REHOVOT 76101, ISRAEL.
CELL 46 (7). 1986. 1091-1102. CODEN: CELLB
Full Journal Title: Cell
Language: ENGLISH

cDNA clones of the Drosophila epidermal growth factor receptor homolog (DER) gene were isolated and sequenced. The deduced amino acid sequence shows a similar degree of homology to the human epidermal growth factor receptor and to the rat and human neu proteins; the most striking difference is the addition of a third cysteine-rich extracellular domain in DER. The structure of the cDNA indicates the use of alternative 5' exons. Thus, the gene encodes three putative proteins differing at their N termini. The distribution of DER transcripts was analyzed by in situ hybridization. Transcripts are uniformly distributed in embryos, larval transcripts are primarily localized to proliferating tissues of the

imaginal discs and brain cortex, and adult transcripts are detected mainly in the brain and ganglia. All three splicing alternatives show similar tissue distribution during development.

9/7/6 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

6122787 EMBASE No: 86117847
Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor
Yamamoto T.; Ikawa S.; Akiyama T.; et al.
Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108 JAPAN
NATURE (ENGLAND) , 1986, 319/6050 (230-234) CODEN: NATUA
LANGUAGES: ENGLISH
A novel v-erb-B-related gene, c-erb-B-2, which has been identified in the human genome, maps to human chromosome 17 at q21, and seems to encode a polypeptide with a kinase domain that is highly homologous with, but distinct from, that of the epidermal growth factor (EGF) receptor. The c-erb-B-2 gene is conserved in vertebrates and it has been suggested that the neu gene, detected in a series of rat neuro/glioblastomas, is, in fact, the rat c-erb-B-2 gene. Amplification of the c-erb-B-2 gene in a salivary adenocarcinoma and a gastric cancer cell line MKN-7 suggests that its over-expression is sometimes involved in the neoplastic process. To determine the nature of the c-erb-B-2 protein, we have now molecularly cloned complementary DNA for c-erb-B-2 messenger RNA prepared from MKN-7 cells. Its sequence shows that the c-erb-B-2 gene encodes a possible receptor protein and allows an analysis of the similarity of the protein to the EGF receptor and the neu product. As a consequence of chromosomal aberration in MKN-7 cells, a 4.6-kilobase (kb) normal transcript and a truncated 2.3-kb transcript of c-erb-B-2 are synthesized at elevated levels. The latter transcript presumably encodes only the extracellular domain of the putative receptor.

9/7/7 (Item 1 from file: 76)
DIALOG(R)File 76:Life Sciences Collection

01384629 2292190
A truncated, secreted form of the epidermal growth factor receptor is encoded by an alternatively spliced transcript in normal rat tissue.
Petch, L.A.; Harris, J.; Raymond, V.W.; Blasband, A.; Lee, D.C.; Earp, H.S.
Dep. Pharmacol., Lineberger Cancer Res. Cent., Univ. North Carolina, Chapel Hill, NC 27599, USA
MOL. CELL. BIOL. vol. 10, no. 6, pp. 2973-2982 (1990.)
DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH
SUBFILE: Biochemistry Abstracts Part 2: Nucleic Acids; Genetics Abstracts; Biochemistry Abstracts Part 3: Amino Acids, Peptides and Proteins; Oncogenes Abstracts

Two independent cDNA clones corresponding to a 2.7-kilobase (kb) epidermal growth factor receptor (EGF-R) mRNA were isolated from a rat liver cDNA library. Sequence analysis revealed 100% homology in the external domain when compared with the full-length rat EGF-R nucleotide sequence and 80 to 90% similarity relative to the human EGF-R. However, the

3'-terminal sequence of these clones did not match EGF-R or any other known sequence(s) and was distinct from the 3' end of the 2.8-kb mRNA, which encodes a truncated EGF-R in A431 cells. The deduced amino acid sequence revealed an open reading frame which is homologous to the external domain of the EGF-R but which terminates prior to the transmembrane region. Southern blot analysis of rat genomic DNA indicated that the 3'-terminal sequence of this transcript is derived from the EGF-R gene.

9/7/8 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08600883 96222282
Humoral and cellular responses raised against the human HER2 oncoprotein are cross-reactive with the homologous product of the new proto-oncogene, but do not protect rats against B104 tumors expressing mutated neu.
Taylor P; Gerder M; Moros Z; Feldmann M
Center for Experimental Medicine, Venezuelan Institute for Scientific Research, IVIC, Caracas, Venezuela. ptaylor@medicina.ivic.ve
Cancer Immunol Immunother (GERMANY) Mar 1996, 42 (3) p179-84, ISSN 0340-7004 Journal Code: CN3
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The neu proto-oncogene encodes a plasma membrane protein belonging to the epidermal growth factor receptor family. The cell line B104, derived from BDIX rat neuroblastoma, carries a point mutation in neu, and forms a tumor when injected into these rats. The human homologue of the neu oncogene (here called HER2) is overexpressed in certain types of cancer. Rats were immunized with HER2 protein (HER2) to investigate a possible cross-reaction between the homologous proteins which could protect them against subsequent inoculation with B104. Specific antibody in the serum was measured by cell-based enzyme-linked immunoabsorbent assay and fluorescence immunocytochemistry, and delayed-type hypersensitivity by an ear assay. Sera from animals immunized with the HER2 extracellular domain (HER2-ECD) reacted with both HER2- and neu-expressing cells. In the ear assay, a significant cellular response to both HER-ECD ($P < 0.05$) and neu protein ($P < 0.001$) was observed in HER2-ECD-immunized rats. However, the growth of B104 tumors in rats was not affected by preimmunization with HER2-ECD. The results indicate that an autoreactive immune response to neu was induced by immunization with HER2-ECD, but was too weak to affect the growth of the neu-bearing tumor.

9/7/9 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07028238 93203131
The role of erbB-2 and its ligands in growth control of malignant breast epithelium.
Lupu R; Dickson RB; Lippman ME
Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC 20007.
Princess Takamatsu Symp (UNITED STATES) 1991, 22 p49-60, Journal Code: HHI
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL

A wealth of recently derived information has strongly implicated the protooncogene erbB-2 (also termed HER-2 or neu) and its protein product as critically involved in human breast cancer as well as other important epithelial malignancies. Because of its substantial homology with the EGF receptor, erbB-2 has long been assumed to encode a growth factor receptor, although until recently definitive identification of ligand(s) has remained elusive. Both in a mutated form and when overexpressed in a non-mutated form, erbB-2 is capable of inducing malignant transformation of many target cells including immortalized breast epithelium. We have recently identified and purified a 30 kDa size growth factor secreted by some human breast cancer cells. The factor is related to transforming growth factor-alpha (TGF-alpha) in its ability to bind to the epidermal growth factor (EGF) receptor (though with about 10 fold lower apparent affinity), its ability to phosphorylate EGF receptor and its ability to induce cloning of normal rat kidney (NRK) fibroblasts. However, it is distinct from TGF-alpha as determined by peptide mapping and its ability to induce activation of erbB-2. TGF-alpha and EGF are incapable of directly inducing phosphorylation of erbB-2. However, in a variety of spontaneously occurring tumor cells as well as cell lines transfected with erbB-2 prepared in our laboratory, 30 kDa glycoprotein (gp30) is capable of inducing direct phosphorylation of erbB-2. The ability to induce phosphorylation of erbB-2 is not inhibited by an anti-EGF receptor blocking antibody. In cells that overexpress erbB-2, the gp30 low concentrations is stimulatory of both standard mitogenesis assays and in clonogenic assays. At higher concentrations, the ligand is growth inhibitory in both of these assays. Because of the ability of gp30 to compete for binding with antibodies directed against erbB-2 which inhibit growth, the gp30 ligand is capable of reversing antibody-induced inhibition of growth. In addition, the gp30 ligand can overcome inhibitory effects seen in cells which overexpress erbB-2 which are induced by extracellular domain fragments of the erbB-2 receptor, once again suggesting a specific pathway of action for the gp30 ligand mediated for interaction with erbB-2.(ABSTRACT TRUNCATED AT 400 WORDS) (23 Refs.)

9/7/10 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06057472 87002474
Alternative 5' exons and tissue-specific expression of the Drosophila EGF receptor homolog transcripts.
Schejter ED; Segal D; Glazer L; Shilo BZ
Cell (UNITED STATES) Sep 26 1986, 46 (7) p1091-101, ISSN 0092-8674
Journal Code: CQ4
Languages: ENGLISH
Document type: JOURNAL ARTICLE
cDNA clones of the Drosophila epidermal growth factor receptor homolog (DER) gene were isolated and sequenced. The deduced amino acid sequence shows a similar degree of homology to the human epidermal growth factor receptor and to the rat and human neu proteins; the most striking difference is the addition of a third cysteine-rich extracellular domain in DER. The structure of the cDNA indicates the use of alternative 5' exons. Thus, the gene encodes three putative proteins differing at their N termini. The distribution of DER transcripts was analyzed by in situ hybridization. Transcripts are uniformly distributed in embryos, larval transcripts are primarily localized to proliferating tissues of the imaginal discs and brain cortex, and adult transcripts are detected mainly

in the brain and ganglia. All three splicing alternatives show similar tissue distribution during development.

9/7/11 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS FULLTEXT

00598620
ORDER fax of complete patent from KR SourceOne. See HELP ORDER348
Recombinant stimulating factor of the neu receptor.
Rekombinanter, stimulierender Faktor fur den "neu"-Rezeptor.
Facteur stimulant du recepteur "neu" recombinant.
PATENT ASSIGNEE:
AMGEN INC., (923231), 1840 Dehavilland Drive, Thousand Oaks California 91320 -1789, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
YEDA RESEARCH AND DEVELOPMENT COMPANY, LTD., (268940), Weizmann Institute of Science Herzl Street at Yavne Road P.O. Box 95, Rehovot 76100, (IL), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
Wen, Duanzhi, 517 Raindance Street, Thousand Oaks, California 91360, (US)
Peles, Elior, 209 Ben Yehuda Street, Tel Aviv 63502, (IL)
Yarden, Yosef, 7 Hachita Street, Rehovot, (IL)
LEGAL REPRESENTATIVE:
Brown, John David et al (28811), FORRESTER & BOEHMERT Franz-Joseph-Strasse 38, D-80801 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 583050 A2 940216 (Basic)
EP 583050 A3 941005
APPLICATION (CC, No, Date): EP 93303308 930428;
PRIORITY (CC, No, Date): US 877431 920429
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/12; C07K-013/00; A61K-037/02; C12P-021/08; G01N-033/577; C12N-005/10; C12N-001/21; C12N-001/21; C12R-001/19

ABSTRACT EP 583050 A2
Recombinant neu receptor stimulating factor, analogs of the factor, DNA sequences encoding the same, and methods of production are disclosed. Pharmaceutical compositions and methods of treating disorders involving neu receptor expression are also described. (see image in original document)
ABSTRACT WORD COUNT: 41

LEGAL STATUS (Type, Pub Date, Kind, Text):
Application: 940216 A2 Published application (A1with Search Report ;A2without Search Report)
Search Report: 941005 A3 Separate publication of the European or International search report
Examination: 950802 A2 Date of filing of request for examination: 950424
Withdrawal: 960508 A2 Date on which the European patent application was withdrawn: 960307
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:
Available Text Language Update Word Count

CLAIMS A	(English)	EPABF2	1350
SPEC A	(English)	EPABF2	8449
Total word count - document A			9799
Total word count - document B			0
Total word count - documents A + B			9799

CLAIMS EP 583050 A3

1. A non-naturally-occurring polypeptide comprising an amino acid sequence sufficiently duplicative of that of naturally-occuring neu receptor stimulating factor to allow possession of one or more biological activities of naturally-occurring neu receptor stimulating factor.
2. A polypeptide according to Claim 1 which is a product of procaryotic or eucaryotic expression of an exogenous DNA sequence.
3. A polypeptide according to Claim 2 which is a product of non-human mammalian cell expression.
4. A polypeptide according to Claim 3 in which the non-human mammalian cell is a CHO cell.
5. A polypeptide according to Claim 2 which is a product of E. coli cell expression.
6. A polypeptide according to Claim 2 which is a product of yeast cell expression.
7. A polypeptide according to Claim 2 wherein the exogenous DNA sequence is a cDNA sequence.
8. A polypeptide according to Claim 7 wherein the cDNA sequence is the DNA sequence of Figure 5 (SEQ ID NO:4).
9. A polypeptide according to Claim 2 wherein the exogenous DNA sequence is a genomic DNA sequence which is homologous to the cDNA sequence of Figure 5 (SEQ ID NO:4).
10. A polypeptide according to Claim 2 wherein the exogenous DNA sequence is a manufactured DNA sequence.
11. A polypeptide according to Claim 2 wherein the exogenous DNA sequence is carried on an autonomously replicating DNA plasmid or viral vector.
12. A polypeptide according to claim 1 comprising the amino acid sequence of rat neu receptor stimulating factor set forth in Figure 5 (SEQ ID NO:4), or any genetically engineered variant thereof.
13. A polypeptide according to Claim 1 comprising the amino acid sequence of the EGF-like domain of rat neu receptor stimulating factor set forth in Figure 7 (SEQ ID NO:5).
14. A polypeptide according to Claim 1 comprising the amino acid sequence of the immunoglobulin-like domain of rat neu receptor stimulating factor set forth in Figure 8 (SEQ ID NO:14).
15. A polypeptide according to Claim 1 which has one or more in vivo biological activities of naturally-occurring neu receptor stimulating factor.
16. A polypeptide according to Claim 1 which has one or more in vitro biological activities of naturally-occurring neu receptor stimulating factor.
17. An isolated DNA sequence for use in securing expression in a procaryotic or eucaryotic host cell of a polypeptide product having an amino acid sequence sufficiently duplicative of that of naturally-occurring neu receptor stimulating factor to allow possession of one or more biological activities of naturally occurring neu receptor stimulating factor, said DNA sequence selected from among:

(a) the DNA sequences set out in Figures 4 and 5 (SEQ ID NO:3 and SEQ ID NO:4, respectively) or their complementary strands;

(b) DNA sequences which hybridize to the DNA sequences defined in (a) or fragments thereof; and
(c) DNA sequences which, but for the degeneracy of the genetic code, would hybridize to the DNA sequences defined in (a) and (b).

18. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to Claim 17 in a manner allowing the host cell to express the polypeptide product.
19. A polypeptide product of the expression of a DNA sequence of Claim 17 in a procaryotic or eucaryotic host cell.
20. An isolated DNA sequence encoding procaryotic or eucaryotic host expression of a polypeptide having an amino acid sequence sufficiently duplicative of that of naturally-occurring neu receptor stimulating factor to allow possession of one or more biological activities of naturally-occurring neu receptor stimulating factor.
21. A cDNA sequence according to Claim 20.
22. A genomic DNA sequence according to Claim 20.
23. A manufactured DNA sequence according to Claim 20.
24. A DNA sequence according to Claim 20 as set out in Figures 4 and 5 (SEQ ID NO:3 and SEQ ID NO:4, respectively).
25. A DNA sequence according to Claim 20 including one or more codons preferred for expression in E.coli cells.
26. A DNA sequence according to Claim 20 including one or more codons preferred for expression in yeast cells.
27. A DNA sequence according to Claim 20 including one or more codons preferred for expression in mammalian cells.
28. A biologically functional plasmid or viral DNA vector including a DNA sequence according to Claim 20.
29. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to Claim 20.
30. A polypeptide product of the expression in a procaryotic or eucaryotic host cell of a DNA sequence according to Claim 20.
31. A DNA sequence encoding a polypeptide fragment or polypeptide analog of naturally-occurring neu receptor stimulating factor.
32. A DNA sequence as in Claim 31 encoding methionyl neu receptor stimulating factor.
33. A polypeptide having part or all of the amino acid sequence set forth in Figure 5 (SEQ ID NO:4) and having one or more of the in vitro or in vivo biological activities of naturally-occurring neu receptor stimulating factor.
34. A polypeptide having part or all of the secondary structure of naturally-occurring neu receptor stimulating factor and having part or all of the amino acid sequence set forth in Figure 5 (SEQ ID NO:4), and having one or more biological properties of naturally-occurring neu receptor stimulating factor.
35. A DNA sequence coding for an analog of human neu receptor stimulating factor selected from the group consisting of:
a) (Met-1) neu receptor stimulating factor; and
b) neu receptor stimulating factor wherein one or more cysteines are replaced by alanine or serine.
36. A polypeptide product of the expression in a procaryotic or eucaryotic host cell of a DNA sequence according to Claim 35.
37. A non-naturally-occurring polypeptide having one or more biological activities of naturally-occurring neu receptor stimulating factor, said polypeptide having an amino acid sequence set forth in Figure 5 (SEQ ID NO:4), or any derivatives, deletion analogs, substitution analogs, or addition analogs thereof, and characterized by being the product of procaryotic or eucaryotic expression of an exogenous DNA sequence.

38. A process for the production of neu receptor stimulating factor comprising:

growing, under suitable nutrient conditions, procaryotic or eucaryotic host cells transformed or transfected with a DNA according to Claim 17, and

isolating the desired polypeptide product of the expression of the DNA sequence in said vector.

39. A method of modulating cellular proliferation and differentiation, comprising contacting the cells with an effective amount of recombinant neu receptor stimulating factor.
40. A method according to Claim 39 which is carried out in a mammal.
41. A method according to Claim 40 in which the mammal is a human.
42. A method for enhancing repair and regeneration of human tissues that express the neu receptor, comprising administering an effective amount of recombinant neu receptor stimulating factor.
43. A method according to Claim 42, in which the tissues are selected from the group consisting of the gastrointestinal, respiratory, urinary and reproductive tract tissues.
44. A method according to Claim 42, in which the tissues comprise human skin.
45. A method according to Claim 42, in which the tissues are neural tissues.
46. A method of treating neu receptor stimulating factor deficiencies in human tissues that express the neu receptor, comprising administering an effective amount of recombinant neu receptor stimulating factor to a human having such a deficiency.
47. A method of treating a mammalian tumor expressing neu receptor on the surface of the tumor cells, comprising administering to a mammal having such a tumor an amount of recombinant neu receptor stimulating factor effective to reduce tumor growth.
48. A method according to Claim 47, wherein the mammal is a human.
49. A method according to Claim 48, used for the treatment of carcinoma selected from the group consisting of prostate, ovary, breast, stomach, lung, kidney and skin carcinomas.
50. A pharmaceutical composition comprising an effective amount of recombinant neu receptor stimulating factor and a pharmaceutically-acceptable diluent, adjuvant or carrier.
51. An antibody specifically generated by immunization with a polypeptide according to Claim 12.
52. An antibody according to Claim 51 which is a monoclonal antibody.
53. A biologically active composition comprising the polypeptide of Claim 1 covalently attached to a water soluble polymer.
54. A composition according to Claim 53 in which the polymer is selected from the group consisting of polyethylene glycol and copolymers of polyethylene glycol and polypropylene glycol.
55. A method of detecting underexpression or overexpression of neu receptor stimulating factor mRNA in human cells or tissues, comprising:

(a) isolating RNA from the cells or tissues;

(b) contacting the RNA with a nucleic acid probe capable of hybridizing with a nucleic acid sequence present in neu receptor stimulating factor mRNA under conditions appropriate for hybridization of the probe with the nucleic acid for which it is specific; and

(c) determining the level of hybridization between the RNA and the probe, as indicative of underexpression or overexpression of the neu receptor stimulating factor mRNA.

9/7/12 (Item 2 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS FULLTEXT

00595097
ORDER fax of complete patent from KR SourceOne. See HELP ORDER348
HER4, a human receptor tyrosine kinase of the epidermal growth factor receptor family.
HER4, menschlicher Rezeptor Tyrosine Kinase von der epidermalen Wachstumsfaktor Rezeptor Familie.
HER4, recepteur tyrosine kinase de la famille du recepteur de facteur de croissance epidermique.
PATENT ASSIGNEE:
Bristol-Myers Squibb Company, (205414), 345 Park Avenue, New York, N.Y. 10154, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
Plowman, Gregory D., 1000 Union Street No. C., Seattle, WA 98101, (US)
Culouscou, Jean-Michel, 3034 W. Viewmont Way W., Seattle, WA 98199, (US)
Shoyab, Mohammed, 2405 Westmont Way W., Seattle, WA 98199, (US)
LEGAL REPRESENTATIVE:
Kinzebach, Werner, Dr. et al (6468), Patentanwalte Reitstotter, Kinzebach und Partner Postfach 86 06 49, D-81633 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 599274 A1 940601 (Basic)
APPLICATION (CC, No, Date): EP 93118837 931123;
PRIORITY (CC, No, Date): US 981165 921124
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/12; C07K-013/00; C12P-021/08; C12N-005/10; G01N-033/68; G01N-033/577; A61K-039/395;

ABSTRACT EP 599274 A1
The molecular cloning, expression, and biological characteristics of a novel receptor tyrosine kinase related to the epidermal growth factor receptor, termed HER4/p180(sup(erbB4,) are described. A HER4 ligand capable of inducing cellular differentiation of breast cancer cells is also disclosed. In view of the expression of HER4 in several human cancers and in certain tissues of neuronal and muscular origin, various diagnostic and therapeutic uses of HER4-derived and HER4-related biological compositions are provided. (see image in original document)
ABSTRACT WORD COUNT: 80

LEGAL STATUS (Type, Pub Date, Kind, Text):
Application: 940601 A1 Published application (A1with Search Report ;A2without Search Report)
Examination: 941102 A1 Date of filing of request for examination: 940902
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF2	942
SPEC A	(English)	EPABF2	14589
Total word count - document A			15531
Total word count - document B			0
Total word count - documents A + B			15531

CLAIMS EP 599274 A1

1. A recombinant polynucleotide comprising a sequence of at least about 200 nucleotides having greater than 80 % homology to a contiguous portion of the HER4 nucleotide sequence depicted in FIG. 1 or its complement.
2. The recombinant polynucleotide of claim 1 comprising a sequence of nucleotides encoding at least about 70 contiguous amino acids within the HER4 amino acid sequence depicted in FIG. 1.
3. The recombinant polynucleotide of claim 1 comprising a contiguous sequence of at least about 200 nucleotides within the HER4 nucleotide coding sequence depicted in FIG. 1 or its complement.
4. The recombinant polynucleotide of claim 1 comprising the HER4 nucleotide coding sequence depicted in FIG. 1 or its complement.
5. A recombinant polynucleotide which encodes a polypeptide having structural characteristics equivalent to that of HER4, which polynucleotide is obtained by single or multiple base addition, deletion and/or substitution in a nucleotide sequence of one of the claims 1 to 4, or which is obtained by selective hybridization with a nucleotide sequence of one of the claims 1 to 4.
6. A recombinant polynucleotide according to one of the claims 1 to 5 which is a DNA polynucleotide.
7. A recombinant polynucleotide according to one of the claims 1 to 5 which is a RNA polynucleotide.
8. An assay kit comprising a recombinant polynucleotide according to one of the claims 1 to 5 to which a detectable label has been added.
9. A polymerase chain reaction (PCR) kit comprising a pair of primers capable of priming cDNA synthesis in a PCR reaction, wherein each primer is a polynucleotide according to claim 6.
10. The PCR kit according to claim 9 further comprising a polynucleotide probe capable of hybridizing to a region of the HER4 gene between and not including the nucleotide sequences to which the primers hybridize.
11. A polypeptide comprising a sequence of at least about 80 amino acids having greater than 90 % identity to a contiguous portion of the HER4 amino acid sequence depicted in FIG. 1.
12. A HER4 polypeptide comprising
    - the amino acid sequence depicted in FIG. 1 from amino acid residues 1 through 1308, or
    - the amino acid sequence depicted in FIG. 1 from amino acid residues 26 through 1308; or
    - the amino acid sequence depicted in FIG. 1 from amino acid residues 1 through 1045; or
    - the amino acid sequence depicted in FIG. 1 from amino acid residues 26 through 1045; or
    - the amino acid sequence depicted in FIG. 2A, or
    - the amino acid sequence depicted in FIG. 1 from amino acid residues 772 through 1308; or
    - the amino acid sequence depicted in FIG. 2B.
13. A polypeptide having structural and/or functional features equivalent to HER4, obtainable by single or multiple amino acid addition, deletion and/or substitution in a sequence of one of the claims 11 or 12.
14. An antibody capable of inhibiting the interaction of a soluble polypeptide and human HER4.
15. An antibody according to claim 14 wherein the soluble polypeptide is a heregulin.
16. An antibody capable of
    a) stimulating HER4 tyrosine autophosphorylation; or
    b) inducing a HER4-mediated signal in a cell, which signal results in

modulation of growth and/or differentiation of the cell; or
c) inhibiting HepG2 fraction 17-stimulated tyrosine phosphorylation of HER4 expressed in CHO/HER4 21-2 cells as deposited with the ATCC (accession number CRL 11205).

17. An antibody which immunospecifically binds to human HER4.
18. An antibody according to claim 17 which
a) resides on the cell surface after binding to HER4; or
b) is internalized into the cell after binding to HER4; or
c) immunospecifically binds to human HER4 expressed in CHO/HER4 21-2 cells as deposited with the ATCC (accession number CRL 11205); or
d) neutralizes HER4 biological activity; or
e) is conjugated to a drug or toxin; or
f) is radiolabeled.
19. Plasmid pBSHER4Y as deposited with the ATCC and having the accession number ATCC 69131.
20. A recombinant vector comprising a nucleotide sequence encoding a polypeptide according to one of the claims 11 to 13.
21. A host cell transfected with a recombinant vector according to claim 20.
22. A recombinant vector comprising a nucleotide sequence encoding a polypeptide according to one of the claims 11 to 13 wherein the coding sequence is operably linked to a control sequence which is capable of directing the expression of the coding sequence in a host cell transfected therewith.
23. A host cell transfected with a recombinant vector according to claim 22.
24. Cell line CHO/HER4 21-2 as deposited with the ATCC and having the accession number CRL 11205.
25. An assay for detecting the presence of a HER4 ligand in a sample comprising:
(a) applying the sample to cells which have been engineered to overexpress HER4; and
(b) detecting an ability of the ligand to affect an activity mediated by HER4.
26. The method according to claim 25, wherein the cells are CHO/HER4 21-2 cells as deposited with the ATCC and having the accession number CRL 11205.
27. The method according to claim 25, wherein the activity detected is HER4 tyrosine phosphorylation, or morphologic differentiation.
28. A ligand for HER4 comprising a polypeptide which binds to HER4,

File Copy

04147058 85061599

Monoclonal antibodies identify a cell-surface antigen associated with an activated cellular oncogene.

Drebin JA; Stern DF; Link VC; Weinberg RA; Greene MI

Nature (ENGLAND) Dec 6-12 1984, 312 (5994) p545-8, ISSN 0028-0836
Journal Code: NSC
Contract/Grant No.: 5-T32-GM07753, GM, NIGMS; CA 14723, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

A variety of antigens have been identified on the surface of the malignant cell. However, identical antigens are often found on non-malignant cells of the same or different histological origin, or of a different stage of embryonic development. Many of these tumour-associated antigens appear to be only incidentally expressed on neoplastic cells. Clearly, it would be of great interest to identify cell-surface antigens whose expression is associated specifically with the transformed state and linked directly with the mechanisms responsible for transformation. The detection of activated cellular oncogenes in human and animal cancer cells by the technique of DNA transfection has allowed the isolation of genetic elements which are thought to have a critical role in malignancy. Here, in an effort to identify cell-surface antigens associated with the neoplastic process, we have generated hybridomas which secrete monoclonal antibodies that react specifically with cell-surface determinants found on NIH 3T3 cells transformed by transfection with a group of rat neuroblastoma oncogenes. These antibodies bind to and immunoprecipitate a phosphoprotein of relative molecular mass 185,000 (185 K) from a DNA donor rat neuroblastoma and 13 independent rat neuroblastoma DNA transfectants. There was no antibody reactivity with normal NIH 3T3 cells or with NIH 3T3 cells transformed by various other agents.

?

102(b) for claims 13, 15

103 for remaining claims

Still need checking

08927150 97146463

Sterically stabilized anti-HER2 immunoliposomes: design and targeting to human breast cancer cells in vitro.

Kirpotin D; Park JW; Hong K; Zalipsky S; Li WL; Carter P; Benz CC; Papahadjopoulos D

Department of Cellular and Molecular Pharmacology, University of California, San Francisco 94143, USA. dkizpo@itsa.ucsf.edu

Biochemistry (UNITED STATES) Jan 7 1997, 36 (1) p66-75, ISSN 0006-2960 Journal Code: A0G

Contract/Grant No.: P50CA58207, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Liposomes (70-100 nm) of 1-palmitoyl-2-oleoylphosphatidylcholine, cholesterol, and poly(ethylene glycol) (PEG)-modified phosphatidylethanolamine (PEG-DSPE) were conjugated to Fab' fragments of a humanized recombinant MAb against the extracellular domain of HER2/neu to create sterically stabilized immunoliposomes (anti-HER2 SL) as a drug carrier targeting HER2-overexpressing cancers. Conjugation employed maleimide-terminated membrane-anchored spacers of two kinds: a short spacer, providing attachment of Fab' close to the liposome bilayer, or a long spacer, with Fab' attachment at the distal terminus of the PEG chain. Confocal microscopy and spectrofluorometry of HER2-overexpressing breast cancer cells incubated with fluorescently labeled anti-HER2 SL prepared with either spacer showed binding of liposomes (8000-23000 vesicles/cell) followed by endocytosis (rate constant ke = 0.012-0.033 min-1) via the coated-pit pathway, evidenced by intracellular acidification and colocalization with transferrin. Uptake of anti-HER2 immunoliposomes by breast cancer cells with low HER2 expression, or after preincubation of cells with free anti-HER2 Fab', was less than 0.2% and 4.3%, respectively, of the uptake by HER2-overexpressing cells. Increasing PEG-DSPE content (up to 5.7 mol %) in anti-HER2-SL prepared with the short spacer decreased liposome-cell binding affinity 60-100-fold, while ke decreased only 2-fold; however, when Fab' fragments were conjugated via a PEG spacer, both binding affinity and ke were unaffected by PEG-DSPE content. Cell binding and internalization of anti-HER2 immunoliposomes increased at higher surface density of conjugated Fab' fragments, reaching plateaus at approximately 40 Fab'/liposome for binding and approximately 10-15 Fab'/liposome for internalization. Uptake of anti-HER2 immunoliposomes correlated with the cell surface density of HER2 and significantly ($p < 0.005$) correlated with the antiproliferative effect of the targeting antibody but not with the total level of cellular HER2 expression. The results obtained were used to optimize in vivo preclinical studies of anti-HER2 SL loaded with antineoplastic drugs.

9/7/2

08591633 96223704

erbB family receptor expression and growth regulation in a newly isolated human breast cancer cell line.

Ethier SP; Kokeny KE; Ridings JW; Dilts CA

Department of Radiation Oncology, University of Michigan Medical School, Ann Arbor 48109-0582, USA.

Cancer Res (UNITED STATES) Feb 15 1996, 56 (4) p899-907, ISSN 0008-5472 Journal Code: CNF

Contract/Grant No.: CA40064, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

A new human breast cancer cell line (SUM-52PE), originating from a malignant pleural effusion specimen, that can be cultured under serum-free conditions has been isolated. Experiments were conducted to examine the relationship between expression of the erbB family of growth factor receptors and growth regulation in these cells. SUM-52PE cells are epidermal growth factor receptor negative but express single copy levels of erbB-2 protein. Southern blot analysis indicates that the erbB-2 gene is not amplified in these cells. The cells also express mRNA for both erbB-3 and erbB-4. Phosphotyrosine Western blot analysis of membrane protein obtained from SUM-52PE cells indicates the presence of a constitutively tyrosine phosphorylated M(r) 185,000 protein. Immunoprecipitation, using antibodies to erbB-2 or erbB-3, coupled to phosphotyrosine Western blot analysis indicates that both erbB-2 and erbB-3 are constitutively tyrosine phosphorylated in proliferating SUM-52PE cells. Conditioned medium obtained from SUM-52PE cells does not induce tyrosine phosphorylation of p185erbB-2 in a sensitive indicator cell line, suggesting that an erbB-2 activating factor is not secreted by these cells. However, neu differentiation factor/heregulin (NDF/HRG) mRNA is expressed by the cells, and Western blot analysis of SUM-52PE membrane protein revealed the presence of a M(r) 90,000 immunoreactive NDF/HRG protein. Thus, SUM-52PE cells synthesize a membrane bound form of NDF/HRG that may activate erbB-2 and erbB-3 via a juxtacrine mechanism. The addition of exogenous beta-2-NDF/HRG to the culture medium of SUM-52PE cells yields enhanced tyrosine phosphorylation of p185erbB-2/erbB-3 but has only a small stimulatory effect on the proliferation of these cells. By contrast, an erbB-2 monoclonal antibody that binds to the extracellular domain of erbB-2 is potently mitogenic for these cells. SUM-52PE cells were also found, by phosphotyrosine Western blot analysis, to express an inordinately large number of tyrosine phosphoproteins. Direct measurement of phosphotyrosine phosphatase (PTPase) activity in SUM-52PE cell membrane protein revealed 2-3-fold lower levels of PTPase activity compared to other normal and neoplastic breast epithelial cell lines. Thus, SUM-52PE cells exhibit altered growth phenotypes not identified previously in human breast cancer cells. The constitutive activation of erbB-2 and erbB-3 in these cells, coupled with their low, membrane-associated, PTPase activity are likely to play direct roles in driving proliferation of these breast cancer cells.

9/7/3
DIALOG(R)File 155:MEDLINE(R)

08475329 96075411

Radioimmunotherapy of breast cancer xenografts with monoclonal antibody ICR12 against c-erbB2 p185: comparison of iodogen and N-succinimidyl 4-methyl-3-(tri-n-butylstannyl)benzoate radioiodination methods.

Smellie WJ; Dean CJ; Sacks NP; Zalutsky MR; Garg PK; Carnochan P; Eccles SA
Department of Surgery, Royal Marsden Hospital, Sutton, Surrey, United Kingdom.

Cancer Res (UNITED STATES) Dec 1 1995, 55 (23 Suppl) p5842s-5846s, ISSN 0008-5472 Journal Code: CNF
Contract/Grant No.: CA 42324, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

C-erbB2 p185 is a proto-oncogene product expressed in 25-30% of human invasive breast cancers that is associated with poor prognosis and resistance to endocrine therapy and chemotherapy. It is minimally expressed in normal adult tissues (M. F. Press et al., Oncogene, 5: 953-962, 1990). For this reason, it is an attractive target for radioimmunotherapy and other antibody-directed therapies. ICR12 is a rat IgG2a monoclonal antibody directed against a protein epitope of the external domain of the c-erbB2 p185. We performed experiments to optimize the direct iodination of ICR12 with 131I using the IodoGen method, and we found impairment of immunoreactive fraction with increasing specific activity. N-Succinimidyl 4-methyl-3-(tri-n-butylstannyl)benzoate (MATE) is a tin ester that can be radioiodinated easily and then coupled to the epsilon-amino group of lysine residues. This method has been shown to have improved uptake in tumors compared with antibody labeled by direct iodination (P. K. Garg et al., Nucl. Med. Biol., 20: 379-387, 1993). ICR12 could be labeled up to 16 mCi/mg by this technique without loss of immunoreactive fraction. Whole-body retention of MATE-labeled ICR12 was less than IodoGen ($P < 0.0001$). Radioimmunotherapy experiments in athymic mice bearing established MDA MB 361 human breast cancer xenografts showed growth inhibition for > 24 days at a dose of 600 microCi/mouse ($P < 0.0001$) when labeled by the IodoGen technique, and 12 days using the MATE method ($P < 0.0001$).

9/7/4
DIALOG(R)File 155:MEDLINE(R)

08390626 95045940
Antigenic and immunogenic mimicry of the HER2/neu oncoprotein by phage-displayed peptides.
Orlandi R; Menard S; Colnaghi MI; Boyer CM; Felici F
Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy.
Eur J Immunol (GERMANY) Nov 1994, 24 (11) p2868-73, ISSN 0014-2980
Journal Code: EN5
Languages: ENGLISH
Document type: JOURNAL ARTICLE
To recover peptides that antigenically and immunogenically mimic the p185HER2 oncoprotein, we selected the phage-peptide libraries pVIII-9aa and pVIII-9aa. Cys using murine monoclonal antibodies (mAb) MGr2 and MGr6, directed against two distinct epitopes of the p185HER2 extracellular domain. Phage-displayed peptides containing consensus amino acid motifs were recovered and shown to compete specifically for mAb binding on tumor cells that overexpress p185HER2. The deduced amino acid sequence of the peptides suggests that both epitopes defined by the mAb on p185HER2 are discontinuous and that hydrophobic interactions are involved in binding with the mAb. A phage clone displaying the GPLDSLFAQ peptide elicited a specific immune response against the p185HER2 in BALB/c mice, demonstrating that this phage-displayed peptide represents an immunological equivalent of the MGr2 epitope on p185HER2 and might be used as a substitute for this oncoprotein in in vitro and in vivo immunological studies.

9/7/5
DIALOG(R)File 155:MEDLINE(R)

08385394 94320906
Keratinocyte induced chemotaxis in the pathogenesis of Paget's disease of

the breast.
de Potter CR; Eeckhout I; Schelfhout AM; Geerts ML; Roels HJ
N. Goormaghtigh Institute of Pathology, University Hospital Ghent, Belgium.
Histopathology (ENGLAND) Apr 1994, 24 (4) p349-56, ISSN 0309-0167 Journal Code: GB4
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In Paget's disease of the breast, the epidermis contains large clear neoplastic cells. To explain the pathogenesis of this disease, the immunohistochemical characteristics of these cells were investigated in 25 patients. The cytoplasmic presence of low molecular weight cytokeratin and the absence of high molecular weight cytokeratin in all cases confirmed the glandular origin of the Paget cells. Membrane over-expression of the neu-protein was established in 96% of cases. It was hypothesized that epidermal keratinocytes release a chemotactic factor which attracts neu-over-expressing breast carcinoma cells by chemotaxis into the epidermis. The biological assays showed that normal keratinocytes release one or more chemotactic factor(s) into their conditioned medium, which induced spreading and motility of neu-over-expressing SK-BR-3 human breast cancer cells. The conditioned medium of keratinocytes also attracted the SK-BR-3 cells by chemotaxis in a modified Boyden chamber. Furthermore, MCF-7 human breast cancer cells, which do not over-express the neu-protein, were not attracted by chemotaxis of conditioned medium of human keratinocytes. The involvement of the neu-protein in spreading, motility and chemotaxis is further indicated by the inhibition of these processes by monoclonal antibodies against the extracellular domain of the neu-protein. We conclude, therefore, that the Paget cells spread through the epidermis due to the motility induced by a chemotactic factor, which is released by epidermal keratinocytes and whose influence is mediated by the neu-protein.

9/7/6
DIALOG(R)File 155:MEDLINE(R)

08033458 95007524
Regression of established breast carcinoma xenografts with antibody-directed enzyme prodrug therapy against c-erbB2 p185.
Eccles SA; Court WJ; Box GA; Dean CJ; Melton RG; Springer CJ
CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey, United Kingdom.
Cancer Res (UNITED STATES) Oct 1 1994, 54 (19) p5171-7, ISSN 0008-5472 Journal Code: CNF
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The enzyme carboxypeptidase G2 (CPG2) was conjugated to the rat IgG2a monoclonal antibody (mAb) ICR12, which recognizes the external domain of the human c-erbB2 protooncogene product. The conjugate retained antigen-binding and enzyme activity. Radiolabeled conjugate localized efficiently and stably to MDA MB 361 breast carcinoma xenografts, which overexpress the c-erbB2 gene product p185. Radiotracer determinations and plasma enzyme activity studies in nu/nu mice gave conjugate blood clearance rate half-lives of approximately 4 days. In separate antibody-directed enzyme prodrug therapy regimes, one dose of the 4-[(2-chloroethyl)(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid prodrug was administered to nu/nu mice bearing established MDA MB 361 tumors (mean volume, 170-260 mm3). In mice which had received ICR12-CPG2 12-14 days

previously, sustained dose-dependent tumor stasis or regressions were effected, which in some cases persisted throughout observation periods of up to 90 days. In control mice which had received the isotype-matched irrelevant mAb ICR16-CPG2 conjugate, tumors grew progressively, as did those in mice treated with prodrug alone, or treated simultaneously with ICR12-CPG2 and prodrug at the maximum tolerated dose. Control chemotherapy with conventional drugs proved toxic and induced only minimal growth delays.

9/7/7
DIALOG(R)File 155:MEDLINE(R)

08020631 94186530
Elevated serum levels of a c-erbB-2 oncogene product in ovarian cancer patients and in pregnancy.
Meden H; Marx D; Fattahi A; Rath W; Kron M; Wuttke W; Schauer A; Kuhn W
Department of Obstetrics and Gynecology, University of Gottingen, Germany.
J Cancer Res Clin Oncol (GERMANY) 1994, 120 (6) p378-81, ISSN 0171-5216 Journal Code: HL5
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Amplification of the proto-oncogene c-erbB-2 (HER-2/neu) has been shown to be a prognostic marker in ovarian cancer. In order to obtain further information on the biological role of the c-erbB-2 gene product p185 it is necessary to quantify expression levels. In this study we evaluated an enzyme-linked immunosorbent assay (ELISA) for the extracellular domain of p185 to determine whether a soluble oncoprotein fragment can be detected in the serum of ovarian cancer patients and in the serum of pregnant women. Sera from 199 women (57 previously untreated ovarian cancer patients, 62 pregnant women and 80 healthy controls) were assayed in a sandwich ELISA utilizing two mouse monoclonal antibodies. To study c-erbB-2 overexpression in ovarian cancer tissue samples we have used an immunohistochemical technique involving a monoclonal antibody specifically reactive with the external domain of the protein p185. The mean serum value for the normal controls was 1203 HNU/ml with a standard deviation (SD) of 279 HNU/ml and a range of 595-1947 HNU/ml. We chose a level of 1761 HNU/ml (2 SD above the mean) as a cut-off to distinguish individuals with elevated levels. The ovarian cancer patients' serum values ranged from 526 to 16,332 HNU/ml. Immunohistochemically detectable p185 was noted in 8 of 57 ovarian cancer patients. The oncoprotein fragment levels in the sera from these 8 patients ranged from 878 to 16,332 HNU/ml. Of 8 patients with p185 overexpression in their tumors, 4 had elevated serum levels. In the sera from the 49 cancer patients without overexpression the values were distributed in the range 526-2892 HNU/ml. There was no association between serum oncoprotein fragment levels and tumor stage, histological type or grading. Serum concentrations of the p185 fragment in pregnancy ranged from 612 to 3265 HNU/ml. The highest levels were found in the third trimester. The results of the present study raise the possibility that the soluble c-erbB-2 protein level in serum is an indicator for cell proliferation and therefore deserves further evaluation as a diagnostic tool in ovarian cancer patients and pregnancy.

9/7/8
DIALOG(R)File 155:MEDLINE(R)

08020029 94163634
Ligand-like effects induced by anti-c-erbB-2 antibodies do not correlate with and are not required for growth inhibition of human carcinoma cells.
Shawver LK; Mann E; Elliger SS; Dugger TC; Arteaga CL
Department of Cell Biology and Immunology, Berlex Biosciences, Richmond, California 94804.
Cancer Res (UNITED STATES) Mar 1 1994, 54 (5) p1367-73, ISSN 0008-5472 Journal Code: CNF
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The c-erbB-2 gene encodes a M(r) 185,000 tyrosine kinase receptor (p185) with extensive homology to the epidermal growth factor receptor. We have conducted mechanistic studies with several anti-p185 monoclonal antibodies (TAb 250, -255, -257, -260, and -263) directed against the extracellular domain of p185 utilizing the SKBR-3, BT-474, and SKOV-3 cancer cell lines. Several of these antibodies exhibited ligand-mimicking properties: they induced tyrosine phosphorylation of p185; increased the catalytic activity of the receptor substrate phospholipase C-gamma 1; exhibited time- and pH-dependent internalization; induced receptor down-regulation; and increased the turnover of the p185 protein delta 3-fold. However, there was not a universal correlation between the antibody-mediated ligand-like effects and growth inhibition. TAb 250 inhibited BT-474 cells but did not alter p185 phosphotyrosine content or increase receptor turnover in these cells. TAb 260 increased p185 protein turnover but did not affect proliferation of the SKOV-3 cell line. Furthermore, blockade of TAb 250-induced receptor phosphorylation with the tyrosine kinase inhibitor tyrphostin 50864-2 did not abrogate TAb 250-mediated growth inhibition of SKBR-3 cells. These data suggest that ligand-like effects mediated by p185 antibodies are not critical for the growth inhibition of c-erbB-2-overexpressing carcinoma cells.

9/7/9
DIALOG(R)File 155:MEDLINE(R)

08019791 94155127
Prevalence and significance of HER-2/neu expression in early epithelial ovarian cancer.
Rubin SC; Finstad CL; Federici MG; Scheiner L; Lloyd KO; Hoskins WJ
Memorial Sloan-Kettering Cancer Center, New York, New York.
Cancer (UNITED STATES) Mar 1 1994, 73 (5) p1456-9, ISSN 0008-543X Journal Code: CLZ
Contract/Grant No.: CA 52477, CA, NCI; CA 08478, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
BACKGROUND. Although expression of the HER-2/neu oncogene may be of some prognostic importance in advanced ovarian cancer, its role in early-stage disease has not been established. The current study examined the prevalence and significance of HER-2/neu expression in early epithelial ovarian cancer. METHODS. The authors analyzed the expression of HER-2/neu on frozen tumor specimens from 40 patients with early epithelial ovarian cancer using the indirect immunoperoxidase technique with monoclonal antibodies that detect epitopes on the extracellular domain of the HER-2/neu protein. All patients underwent comprehensive surgical staging. HER-2/neu expression was graded as negative, weak, moderate (1+ to 2+), or strong (3+). Complete

clinical data and long-term follow up were available for all patients. RESULTS. The distribution of patients by stage was as follows: Stage IA, 6; IB, 0; IC, 14; IIA, 4; IIB, 6; IIC, 10. The mean patient age was 53 years. Fourteen patients had serous tumors; nine, endometrioid; eight, clear cell; eight, mucinous; and one, undifferentiated. Intratumoral heterogeneity of HER-2/neu expression was observed with most specimens. In eight specimens (20%), some areas of the tumor showed strong (3+) expression, beyond the level that can be seen in normal ovarian epithelium. Twenty-eight specimens (70%) showed moderate (1+ to 2+) staining, whereas four specimens (10%) showed negative or weak staining. At a mean follow-up time among surviving patients of 32 months, 15 patients (37%) have had cancer recurrence. No statistically significant relationship was found between HER-2/neu expression and survival, disease-free survival, stage, or grade. A significant increase was found in 3+ expression of HER-2/neu in clear cell tumors. CONCLUSION. Consistent HER-2/neu overexpression occurs infrequently in early ovarian cancer, making it unlikely that such overexpression is a general early event in ovarian carcinogenesis. HER-2/neu expression does not appear to be a strong prognostic marker in early epithelial ovarian cancer.

9/7/10
DIALOG(R)File 155:MEDLINE(R)

07835222 95196230
Rat MAbs to the product of the c-erbB-2 proto-oncogene for diagnosis and therapy in breast cancer.
Dean CJ; Eccles SA; Valeri M; Box G; Allan S; McFarlane C; Sandle J; Styles J
Institute of Cancer Research, Sutton, Surrey, UK.
Cell Biophys (UNITED STATES) Jan-Jun 1993, 22 (1-3) p111-27, ISSN 0163-4992 Journal Code: CQC
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The product of the c-erbB-2 protooncogene (p185) is a member of the EGF receptor family of transmembrane tyrosine kinases. Amplification of this gene and overexpression of the product has been observed in adenocarcinomas and has been correlated with poor prognosis in patients with breast and ovarian cancer. The very low levels of expression of p185 by normal adult tissues makes the receptor an almost tumor-specific target. We have prepared rat monoclonal antibodies against five distinct epitopes on the external domain of the c-erbB-2 product overexpressed by the breast cancer line BT474. The antibodies bind to the protein core of p185 and stain specifically the membranes in frozen sections of tumors overexpressing the c-erbB-2 product. Three of the antibodies, ICR12 (epitope A), ICR54, and ICR55 (epitope E), also stain the cell membrane in formalin-fixed, paraffin-embedded sections and bind to p185 in Western blots. An investigation of the stability of the antigen-antibody complexes indicates that the majority are not readily internalized by SKOV3 cells growing in vitro. Antibodies ICR12 (IgG2a) and ICR55 (IgG2a), which are directed against separate epitopes on the c-erbB-2 p185, are both of high affinity and immunoreactivity (> 75%) and localize specifically and stably to xenografted breast and ovarian carcinomas growing in athymic mice when labeled with 125I (up to 13% injected dose/g, ICR12 and ICR55) or a range of other radionuclides (up to 20% id/g, ICR12). We conclude that these antibodies may be useful as therapeutic vehicles for targeting radionuclides (imaging and therapy) or enzymes for activating prodrugs

(ADEPT).

9/7/11
DIALOG(R)File 155:MEDLINE(R)

07815514 93154832
Antibody-induced growth inhibition is mediated through immunochemically and functionally distinct epitopes on the extracellular domain of the c-erbB-2 (HER-2/neu) gene product p185.
Xu F; Lupu R; Rodriguez GC; Whitaker RS; Boente MP; Berchuck A; Yu Y; DeSombre KA; Boyer CM; Bast RC Jr
Department of Medicine, Duke University Medical Center, Durham, NC 27710.
Int J Cancer (UNITED STATES) Feb 1 1993, 53 (3) p401-8, ISSN 0020-7136 Journal Code: GQU
Contract/Grant No.: CA 39930, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Over-expression of the c-erbB-2 (HER-2/neu) gene product p185 occurs in 30% of breast and ovarian cancers. The p185 protein might serve as a target for serotherapy in that antibodies against different epitopes on the extracellular domain of p185 can inhibit growth of tumor cells in the absence of cellular or humoral effector mechanisms. To define epitopes of functional relevance, 11 monoclonal antibodies (MAbs) were evaluated for their ability to bind to the extracellular domain of p185. Results of competition studies with 125I-labeled and non-labeled antibodies indicated that 10 of 11 epitopes were grouped in a linear array. Antibodies against 7 epitopes inhibited anchorage-independent growth and antibodies against 2 of these epitopes also inhibited anchorage-dependent growth of SKBr3 breast-cancer cells that over-expressed p185. Treatment with antibodies exerted cytotoxic rather than cytostatic effects. When antibodies were used in combination, additive or supra-additive inhibition of anchorage-independent and anchorage-dependent growth was observed between pairs of antibodies. Growth inhibition did not relate to the affinity of the antibody or its isotype. Two antibodies that inhibited both anchorage-dependent and anchorage-independent growth also blocked binding of the HER-2/neu ligand, whereas 5 antibodies that inhibited only anchorage-independent growth had no effect on ligand binding. Inhibition of cell growth did not correlate with internalization of p185 or down-regulation of p185 on the cell surface. Fab fragments of active antibodies could also inhibit anchorage-independent growth of SKBr3. Thus, murine MAbs and their fragments recognized both immunochemically distinct and functionally distinct epitopes on the p185 molecule. Whereas inhibition of anchorage-dependent growth correlated with the ability of antibodies to block ligand binding, inhibition of anchorage-independent growth did not correlate with effects on ligand binding, internalization, cell-surface expression or cross-linking of p185.

9/7/12
DIALOG(R)File 155:MEDLINE(R)

07811883 95196233
Monoclonal antibodies for the treatment of metastases. Evaluation of strategies using a syngeneic rat model.
Eccles SA; Box G; Court W; Collins MK; Dean CJ

Section of Immunology, Institute of Cancer Research, Sutton, UK.
Cell Biophys (UNITED STATES) Jan-Jun 1993, 22 (1-3) p165-87, ISSN 0163-4992 Journal Code: CQC
Languages: ENGLISH
Document type: JOURNAL ARTICLE

To investigate critical factors influencing the localization and antitumor effects of monoclonal antibodies (MAb) or toxic conjugates, we have adapted a single rat sarcoma, HSN, for preferential growth in the lungs, liver, and lymph nodes (the major sites of metastasis in humans) and have raised a panel of syngeneic rat MAbs to a stably-expressed cell surface antigen. Using this model we have shown that localization in tumors is significantly influenced by their anatomical location and vascularization, and the degree of MAb interaction with host cells. Uptake in small hepatic tumors was excellent, but access to lung tumors was limited by the poor permeability of pulmonary vessels. HSN cells transfected with th human IL-2 gene and coinjected in low numbers with parental tumors secreted sufficient cytokine to enhance the local permeability of vessels and doubled MAb localization in tumors without any systemic toxicity, suggesting that regional delivery of IL-2 may be used to enhance MAb localization in this situation. In order to extent the applicability of the model to studies of MAbs raised against human tumor targets, we have transfected the human c-erb B-2 gene (homolog of the rat neu) into the highly metastatic HSN.LV subline. MAbs raised against the external domain of the p185 product can now be screened for their ability to localize in metastases, and for various conjugates to inhibit tumor growth either independently of, or in association with, a fully functional immune system.

9/7/13
DIALOG(R)File 155:MEDLINE(R)

07801147 94020813
The extracellular domain of the c-erbB-2 oncoprotein is released from tumor cells by proteolytic cleavage.
Pupa SM; Menard S; Morelli D; Pozzi B; De Palo G; Colnaghi MI
Division of Experimental Oncology E, Istituto Nazionale Tumori, Milan, Italy.
Oncogene (ENGLAND) Nov 1993, 8 (11) p2917-23, ISSN 0950-9232 Journal Code: ONC
Languages: ENGLISH
Document type: JOURNAL ARTICLE

A molecule that is immunologically related to the c-erbB-2 oncogene product (p185HER2/neu) was detected in the conditioned culture medium from neu-overexpressing tumor cell lines and in sera of advanced-stage breast carcinoma patients. Using a sensitive (in the range of 0.5 ng ml-1) double-determinant radioimmunoassay (DDIRMA) with two monoclonal antibodies (MAbs) directed against the neu extracellular domain (ECD), soluble oncoproteins were detected in supernatants from several neu-positive tumor cell lines, independent of the levels of membrane p185HER2 expression. The molecule detected did not react with a MAb directed against an intracytoplasmic epitope of the p185HER2. Western blot analysis of the concentrated supernatant revealed a protein of approximately 110 kDa molecular mass, which closely matches the predicted size of the glycosylated p185HER2 ECD. Immunoprecipitation of culture supernatant from cell surface-radioiodinated cells confirmed the 110 kDa molecular mass of the glycosylated shed protein, which migrated to 86 kDa after

deglycosylation. Proteolytic cleavage of the p185HER2 molecule was demonstrated in release assays carried out with protease inhibitors. The combined use of leupeptin and EDTA completely inhibited release of the molecule. Analysis of sera from breast carcinoma patients and healthy donors by DDIRMA revealed the presence of soluble neu in 15% of pathologic sera but none of the normal sera. A good correlation was found between neu-overexpression in the primary tumor and the soluble marker in serum of patients with advanced disease; sera of early-stage patients were always negative, independent of neu-overexpression in the tumor. These results suggest the usefulness of soluble neu as an indicator of tumor aggressiveness but not as a diagnostic marker of breast cancer.

9/7/14
DIALOG(R)File 155:MEDLINE(R)

07798889 93367248
Binding and cytotoxicity characteristics of the bispecific murine monoclonal antibody 2B1.
Weiner LM; Holmes M; Richeson A; Godwin A; Adams GP; Hsieh-Ma ST; Ring DB ; Alpaugh RK
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111.
J Immunol (UNITED STATES) Sep 1 1993, 151 (5) p2877-86, ISSN 0022-1767 Journal Code: IFB
Contract/Grant No.: CA06927, CA, NCI; CA50633, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Bispecific monoclonal antibodies (BsmAb) with specificity for tumor Ag and effector cell trigger molecules have been shown to redirect the cytotoxicity of several peripheral blood mononuclear cell populations against relevant tumor. The BsmAb, 2B1, binds to the extracellular domain of the c-erbB-2 gene product of the HER2/neu proto-oncogene and to CD16. In this report, the binding and cytotoxic characteristics of 2B1 are presented. Maximal saturation binding of 2B1 to PBL and c-erbB-2 expressing SK-OV-3 cells occurred in the 1 microgram/ml concentration range. However, substantial lysis potentiation was observed at 1000-fold lower BsmAb concentrations. Optimal tumor lysis was obtained when the BsmAb, PBL, and target cells were continuously coincubated. When PBL were franked with 2B1, washed, and added to labeled targets, substantially less lysis was observed. These results suggest that the best way to therapeutically exploit the cytotoxic attributes of 2B1 may be to obtain continuous BsmAb exposure to tumor. Approaches based on franking of this BsmAb to PBL may not be warranted.

9/7/15
DIALOG(R)File 155:MEDLINE(R)

07797991 93346103
Characterization of cytotoxic activity of saporin anti-gp185/HER-2 immunotoxins.
Tecce R; Digiesi G; Savarese A; Trizio D; Natali PG
Laboratory of Immunology, Regina Elena Cancer Institute, Rome, Italy.
Int J Cancer (UNITED STATES) Aug 19 1993, 55 (1) p122-7, ISSN 0020-7136 Journal Code: GQU

dimerization of receptor molecules and that bivalency of the activating antibody is mandatory for induction of internalization and phosphorylation of the receptor. Our data support an allosteric model of activation for the p185HER2 receptor.

9/7/17
DIALOG(R)File 155:MEDLINE(R)

07789567 93105424
A human tumor xenograft model of therapy with a bispecific monoclonal antibody targeting c-erbB-2 and CD16.
Weiner LM; Holmes M; Adams GP; LaCreta F; Watts P; Garcia de Palazzo I
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.
Cancer Res (UNITED STATES) Jan 1 1993, 53 (1) p94-100, ISSN 0008-5472 Journal Code: CNF
Contract/Grant No.: CA50633, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
New strategies are required to clinically exploit the ability of monoclonal antibodies to target tumor for lysis by cellular effector mechanisms. In this report we examine the therapeutic effects of 2B1, a bispecific monoclonal antibody with specificity for the extracellular domain of the c-erbB-2 oncogene product and the human Fc gamma receptor, Fc gamma RIII (CD16), describe the characteristics and limitations of this model, and examine the mechanisms underlying the observed responses. The model uses SK-OV-3 human ovarian carcinoma xenografts in scid mice. These cells are susceptible to 2B1-directed lysis by human peripheral blood lymphocytes or lymphokine-activated killer cells, and maintain c-erbB-2 expression in vivo. 125I-labeled 2B1 selectively accumulates in tumor, with a peak of 10.5% injected dose/g of tumor 24 h following its i.v. injection. However, the selectivity of this binding is lessened by 2B1 accumulation in the lungs and other normal organs and persistence in the blood. This is caused by antibody binding to murine lung, colon, stomach, and skin expressing the epitope recognized by the anti-c-erbB-2 component of 2B1 in tumor-bearing, but not normal mice. In treatment studies using various permutations of antibody, human peripheral blood lymphocytes or lymphokine-activated killer cells and interleukin 2, cellular therapy alone had minimal effects on SK-OV-3 xenograft growth, but significantly improved when 2B1 treatment was incorporated. Median survivals increased from 80 +/- 3.5 days with no therapy to 131 +/- 7.3 days following therapy with 100 micrograms 2B1, interleukin 2, and human peripheral blood lymphocytes, with 70% of animals exhibiting no evidence of tumor at day 150. These effects were preserved when the cells were administered in human serum. In contrast, human serum abolished the antitumor effects of 520C9, which is the parent anti-c-erbB-2 antibody of 2B1. Thus 2B1-based therapy has therapeutic effects, without obvious toxicity, despite the targeting of this antibody to normal murine tissues. Since combinations of 2B1 and interleukin 2 may have antitumor properties, mechanisms other than bispecific monoclonal antibody-promoted conjugation of c-erbB-2 antigen-expressing tumor to CD16-expressing effector cells may be involved.

9/7/18
DIALOG(R)File 155:MEDLINE(R)

07487466 93161295
Cell growth regulation in epithelial ovarian cancer.
Bast RC Jr; Boyer CM; Jacobs I; Xu FJ; Wu S; Wiener J; Kohler M; Berchuck A
Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710.
Cancer (UNITED STATES) Feb 15 1993, 71 (4 Suppl) p1597-601, ISSN 0008-543X Journal Code: CLZ
Contract/Grant No.: RO1CA39930, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
BACKGROUND. As in the case of other epithelial neoplasms, most ovarian cancers arise from single clones of cells that have undergone multiple genetic alterations. A comparison of normal and malignant ovarian epithelium has identified several differences in growth regulation by peptide growth factors, protooncogenes, and tumor suppressor genes. METHODS. Recent articles and abstracts have been reviewed. RESULTS. The malignant ovarian epithelial phenotype has been associated with (1) autocrine growth stimulation by transforming growth factor-alpha, (2) loss of autocrine growth inhibition by transforming growth factor-beta, (3) mutation or amplification of ras in 2-12% of cases, (4) amplification of myc in 23% of specimens, (5) expression of fms in 56% of cases with potential autocrine stimulation by macrophage colony stimulating factor, (6) paracrine stimulation by macrophage products including interleukin-1, interleukin-6 and tumor necrosis factor, (7) overexpression of c-erbB-2 (HER-2/neu) in 30% of cases, and (8) mutation with consequent overexpression of p53 in 50% of advanced ovarian cancers. A poor clinical prognosis is associated with expression or overexpression of the epidermal growth factor receptor, fms, and HER-2/neu. Antibodies against the extracellular domain of the HER-2/neu gene product p185 inhibit the growth of tumor cells that overexpress HER-2/neu and are associated with marked decreases in diacylglycerol levels. The intracellular kinase domain is required for growth inhibition. Antibodies that inhibit growth stimulate phosphorylation of intracellular substrates. Ricin A chain monoclonal antibody conjugates that react with p185 also inhibit the growth of tumor cells that overexpress p185. The intracellular kinase region is not required for immunotoxin-mediated killing. Coexpression of HER-2/neu and the epidermal growth factor receptor has been observed in 65% of epithelial ovarian cancers and in a limited number of normal tissue from a fraction of donors. CONCLUSIONS: Multiple alterations in growth factors, protooncogenes and growth factors have been detected in different epithelial ovarian cancers. Inappropriate signalling from receptor tyrosine kinases may be particularly important for ovarian oncogenesis. Drugs that affect tyrosine kinase and phosphatase activity deserve attention as potential therapeutic agents for ovarian cancer. The extracellular domains of the HER-2/neu gene product p185 and the epidermal growth factor receptor may provide useful targets for serotherapy. (55 Refs.)

9/7/19
DIALOG(R)File 155:MEDLINE(R)

07422263 93013814
Production and characterization of murine mAbs to the extracellular domain of human neu oncogene product GP185HER2.
Digiesi G; Giacomini P; Fraioli R; Mariani M; Nicotra MR; Segatto O;

Natali PG
Laboratory of Immunology, Regina Elena Cancer Institute, Rome, Italy.
Hybridoma (UNITED STATES) Aug 1992, 11 (4) p519-27, ISSN 0272-457X
Journal Code: GFS
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The oncogene HER-2/neu encodes a transmembrane glycoprotein of 185 kDa (gp185HER-2) with tyrosine-kinase activity. Gene amplification and high levels of expression of gp185HER-2 have been found to correlate with poor clinical outcome in breast and ovarian carcinomas. Employing a somatic cell hybrid fusion protocol, which yields a high frequency production of hybridomas, we have analyzed the extent of the murine immune response to the gp 185 extracellular domain. In a single fusion experiment, using as immunogen NIH 3T3 cells expressing high levels of a transfected human HER-2 gene, we have generated mAbs, mainly of IgG1 isotype, displaying high affinity (10(7)-10(10) mol/L) to gp 185. Analysis of the epitope specificity has allowed the identification of five distinct groups of spatially related epitopes, each provided with different immunodominancy, and all resistant to formalin fixation. The use of inhibitor of N-linked glycosylation tunicamicyn has demonstrated that the mAbs bind to epitopes localized in the protein core of gp185HER-2. Because recent reports have shown that gp185HER-2 has a restricted expression in normal tissues and is homogenously detectable in metastatic foci of gp 185 + primary tumors, antibodies to this macromolecule, in addition to their prognostic value, may represent reagents for in vitro and in vivo diagnostic applications, as well as for the development of therapeutic strategies.

9/7/20
DIALOG(R)File 155:MEDLINE(R)

07419318 92363416
p185 HER2/neu epitope mapping with murine monoclonal antibodies.
Centis F; Tagliabue E; Uppugunduri S; Pellegrini R; Martignone S; Mastroianni A; Menard S; Colnaghi MI
Division of Experimental Oncology E, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy.
Hybridoma (UNITED STATES) Jun 1992, 11 (3) p267-76, ISSN 0272-457X
Journal Code: GFS
Languages: ENGLISH
Document type: JOURNAL ARTICLE

In order to obtain further information on the biological role of the HER2/neu oncoprotein, 7 new monoclonal antibodies (MAbs) were produced against the p185HER2 extracellular domain. These MAbs, together with two others previously produced, were used to investigate the p185HER2 expression in breast carcinomas and compare the recognized antigenic determinants. The 7 reagents (MGR4,5,6,7,8,9 and 10), were shown to define five distinct epitopes. Three of these MAbs (MGR5,7,10), as well as one previously produced (MGR2), recognize the same epitope (Epitope-1) which seems, therefore, to be highly immunogenic for the murine immune system. Epitope-2 recognized by the MGR4 MAb, appears to be closely related to epitope-1 due to a cross inhibition between MGR4 and MGR10, but not MGR2. Epitope-2 is the only one of the 5 also present on the product of the neu oncogene, the rat analogue of the human HER2/neu gene. None of the reagents against epitope-1 and epitope-2 were found to mediate receptor internalization, whereas MGR6 as well as a previously produced MAb (MGR3), both of which define epitope-3 and MGR8 which defines epitope-4, were found

to do so. Epitope-4 like the neu-specific peptide recognized by the reference c-neu Ab3 MAb, was detectable on all p185HER2 positive breast cancer, independently from the quantitative content of the oncoprotein, at variance with the other 4 epitopes whose availability on p185HER2 for the relevant MAbs varied with the degree of overexpression. Epitope-5, recognized by the MGR9 MAb, on the contrary to the other epitopes, was prevalently localized at the basal membrane level of the tumor nodule.

9/7/21
DIALOG(R)File 155:MEDLINE(R)

07410199 92098213
Effects of interferons on the expression of the proto-oncogene HER-2 in human ovarian carcinoma cells.
Marth C; Cronauer MV; Doppler W; Ofner D; Ullrich A; Daxenbichler G
Department of Obstetrics and Gynecology, Innsbruck University Clinic, Austria.
Int J Cancer (UNITED STATES) Jan 2 1992, 50 (1) p64-8, ISSN 0020-7136 Journal Code: GQU
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The over-expression of the proto-oncogene HER-2 (c-erbB-2/neu) in ovarian, endometrial and mammary carcinoma is an important indicator for poor prognosis. We have previously shown in 3 out of 4 ovarian carcinoma cell lines an interferon-gamma (IFN-gamma)-mediated reduction in HER-2 specific protein and RNA levels. The oncogene expression was lowered only in the ovarian carcinoma cell lines but not in 3 IFN-gamma-sensitive human breast cancer cell lines. We extended our observations also to IFN type I, alpha and omega. The expression of the oncogene was measured by both the p185HER-2 ELISA and in selected cases by a living cell radioimmunoassay using the monoclonal antibody (MAb) 4D5 against the extracellular domain. Both IFN types reduced the expression of HER-2 in the ovarian carcinoma cell lines OVCAR-3, HTB-77, 2774 and SKOV-6, and in the SKUT-2 endometrial carcinoma cells. In contrast, SKOV-8 human ovarian carcinoma cells were sensitive for both IFN types regarding proliferation, but only IFN-gamma reduced proto-oncogene expression. In the SKBR-3 human mammary carcinoma cells, neither IFN type had an effect on HER-2 expression. The antibodies 4D5, 7C2, 3E8, and 3H4 which bind to the extracellular domain of p185HER-2 protein specifically inhibited anchorage-independent growth of SKBR-3 and HTB-77 cells. Expression of the oncogene HER-2 is the leading prognostic factor in ovarian cancer. Its modulation might represent a mechanism by which IFNs inhibit cell proliferation.

9/7/22
DIALOG(R)File 155:MEDLINE(R)

07404418 94218357
c-erbB-2 (HER-2/neu) oncopeptide immunoreactivity in localized, high-grade transitional cell carcinoma of the bladder.
Swanson PE; Frierson HF Jr; Wick MR
Lauren V. Ackerman Laboratory of Surgical Pathology, Washington University School of Medicine, St. Louis, Missouri.
Mod Pathol (UNITED STATES) Sep 1992, 5 (5) p531-6, ISSN 0893-3952 Journal Code: PTH

Languages: ENGLISH
Document type: JOURNAL ARTICLE
Monoclonal antibodies to the extracellular domain of the c-erbB-2 oncoprotein (p185) react with routinely processed, paraffin-embedded human tissues, and positive staining with these reagents has been shown to correlate with gene overexpression. To determine whether such antibodies would add prognostic data to the analysis of a pre-defined subset of transitional cell carcinoma (TCC) of the bladder, we studied 20 high-grade (Grade 3) TCCs from patients known to have limited disease (Jewett-Strong stages B1, B2, and C) and for whom at least 3-yr clinical follow-up was available. Data procured from this immunohistochemical analysis were compared with tumoral DNA content (determined by flow cytometry) and conventional clinicopathologic features. Overall, 13 of 20 TCC (65%) were reactive for p185-erbB-2. However, there was no apparent relationship between p185-reactivity and either DNA content, tumor stage or clinical outcome. These results suggest that c-erbB-2 expression may be augmented in localized high-grade TCC but that there is no evidence for the contention that this phenomenon has any effect on the biologic behavior of these neoplasms. The only factor that predicted a more favorable outcome was a relatively low stage at the time of diagnosis.

9/7/23
DIALOG(R)File 155:MEDLINE(R)

07396562 92399304
The role of erbB-2 and its ligands in growth control of malignant breast epithelium.
Lupu R; Dickson RB; Lippman ME
Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC 20007.
J Steroid Biochem Mol Biol (ENGLAND) Sep 1992, 43 (1-3) p229-36, ISSN 0960-0760 Journal Code: AX4
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
The erbB-2 (HER-2, neu) protooncogene is overexpressed on the surface of about 25% of human breast cancers. It is homologous to epidermal growth factor receptor and a putative growth factor receptor. Overexpression in breast, ovarian and gastric cancers is associated with a worse prognosis. We have recently discovered two ligands for this receptor. They both induce receptor phosphorylation. At low concentrations both induce clonogenic growth of overexpressing cells; at higher concentrations both are growth inhibitory. Both can overcome the inhibitory effects of both monoclonal antibodies directed against the ligand binding site and soluble extracellular domain. These ligands may form an attractive basis for antitumor therapy. (23 Refs.)

9/7/24
DIALOG(R)File 155:MEDLINE(R)

07394851 92364467
[Role of p185c-erbB2 in endometrial cancer growth in vitro]
Sakamoto H; Ohtani K; Ohta H; Takami M; Takami T; Satoh K
Department of Obstetrics and Gynecology, Nihon University, Faculty of Medicine, Tokyo.

Nippon Sanka Fujinka Gakkai Zasshi (JAPAN) Jul 1992, 44 (7) p800-4, ISSN 0300-9165 Journal Code: INR
Languages: JAPANESE Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE English Abstract

Human endometrial adenocarcinoma cells (Ishikawa line) constitutively express c-erbB2 coded oncoprotein p185erbB2 (p185) which is believed to be an orphan receptor for an unknown growth factor. Since we have shown that expression of p185 in primary lesions of endometrial cancer correlates well with high frequency of lymph node++ metastases and that the metastatic cells in the nodes strongly expressed p185, the role of the oncoprotein in processes of metastases was studied. Culturing the cells in the presence of 15% FCS and with monoclonal antibody to the extracellular domain of p185 (CB-1) inhibited cell growth and attenuated p185 expression on Western blotting, whereas no change occurred with the control antibody. Cells cultured without FCS achieved only approximately 1/3 growth compared to cells with FCS, and further suppression of growth was observed after adding CB-1. When cells were cultured on human term amnion, basement membrane invasion with p185 expression was observed. In nude mice, intraperitoneal seeding resulted in implant formation which was also associated with positive p185 as well as cyclin immunohistochemistry. In the two experiments, treatment of cells with CB-1 inhibited invasion or implant formation. The present study suggests that a signal through p185 receptor molecules acts as a trigger for early proliferation, and interaction with the host may enhance up-regulation of p185.

9/7/25
DIALOG(R)File 155:MEDLINE(R)

07236783 93013142
Oncogenes and onco-suppressor gene in adenocarcinoma of the oesophagus.
Jankowski J; Coghill G; Hopwood D; Wormsley KG
Department of Medicine, University of Dundee.
Gut (ENGLAND) Aug 1992, 33 (8) p1033-8, ISSN 0017-5749
Journal Code: FVT
Languages: ENGLISH
Document type: JOURNAL ARTICLE

While the activation of the proto-oncogenes has been implicated in the development and progression of cancer of many tissues, the role of oncogenes in the development of oesophageal adenocarcinoma has not been defined. Fifteen patients who had undergone resection for oesophageal adenocarcinoma and 15 who had undergone oesophagectomy or biopsy for Barrett's oesophagus were studied. The latter patients also had adjacent normal gastric mucosa biopsied for comparison with the metaplastic oesophageal mucosa. The mucosal samples were snap frozen and subsequently stained with monoclonal antibodies to the following oncogene associated proteins; c-erbB2 (neu and CE-1) (external domain), c-erbB2 (NCL-CB11) (internal domain), c-src, c-ras, c-myc, c-fos, c-jun, and the onco-suppressor gene--p53. All tumours were well or moderately differentiated adenocarcinomas arising from the lower third of the oesophagus. Eleven specimens showed strong membraneous staining with both c-erbB2 (neu) and c-erbB2 (CBL-CB11). Seven specimens showed strong nuclear staining with p53 onco-suppressor gene. Three specimens were positive for c-ras and c-src, and two were positive for c-jun. In Barrett's epithelium, nine specimens were positive for c-erbB2 (neu and CB11), three were positive for c-src, two were positive for c-ras and c-jun, and one was positive for c-fos. Two of the gastric mucosal biopsy specimens expressed

c-erbB2 weakly but no other oncogenes were found. The frequency of positive staining for c-erbB2 is very high, compared with the expression of these genes in other tumours.(ABSTRACT TRUNCATED AT 250 WORDS)

9/7/26
DIALOG(R)File 155:MEDLINE(R)

07029176 90150117
Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product.
Fendly BM; Winget M; Hudziak RM; Lipari MT; Napier MA; Ullrich A
Department of Medicinal and Analytical Chemistry, Genentech, Inc., South San Francisco, California 94080.
Cancer Res (UNITED STATES) Mar 1 1990, 50 (5) p1550-8, ISSN 0008-5472 Journal Code: CNF
Languages: ENGLISH
Document type: JOURNAL ARTICLE
High levels of expression of either the epidermal growth factor receptor or the receptor-like HER2/neu gene product p185HER2 have been observed in a variety of human malignancies. Because of the association of this high level expression with certain human tumors, we have generated a panel of monoclonal antibodies specific for either the epidermal growth factor receptor or p185HER2 to study their structure, function, and antigenic domains in the normal and neoplastic states. We used the epidermoid carcinoma line A431 to generate five monoclonal antibodies which immunoprecipitate the epidermal growth factor receptor. These monoclonal antibodies bind to the extracellular domain of the epidermal growth factor receptor and demonstrate variable effects on epidermal growth factor binding. We used a stably transfected NIH 3T3 cell line expressing the HER2/neu gene to produce and characterize 10 monoclonal antibodies which immunoprecipitate p185HER2. These monoclonal antibodies bind to the extracellular domain of p185HER2 and do not cross-react with the epidermal growth factor receptor. The characteristics and potential applications of these monoclonal antibodies will be discussed.

9/7/27
DIALOG(R)File 155:MEDLINE(R)

07020972 92005459
Requirements for the internalization of a murine monoclonal antibody directed against the HER-2/neu gene product c-erbB-2.
Maier LA; Xu FJ; Hester S; Boyer CM; McKenzie S; Bruskin AM; Argon Y; Bast RC Jr
Department of Medicine, Duke Comprehensive Cancer Center, Duke University Medical Center, Durham, North Carolina 27710.
Cancer Res (UNITED STATES) Oct 1 1991, 51 (19) p5361-9, ISSN 0008-5472 Journal Code: CNF
Contract/Grant No.: 5-R01-CA 39930, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A murine monoclonal antibody, TA1, is directed against an epitope on the extracellular domain of the HER-2/neu (c-erbB-2) gene product. Requirements for TA1-induced internalization of c-erbB-2 have been studied using the SKBr3 human breast cancer cell line and several rat fibroblast cell lines

that express either wild-type or mutant human c-erbB-2. Internalization of TA1 was monitored by assaying protease-resistant uptake of 125I-labeled TA1, by electron microscopy of gold-labeled TA1, and by inhibition of clonogenic growth of cells incubated with TA1 that had been conjugated with blocked ricin. Similar rates of internalization of TA1 were observed in SKBr3 and in rat fibroblasts that expressed human c-erbB-2. The route of endocytosis was the same as that observed with antibodies against other membrane receptors. Anti-c-erbB-2 and anti-transferrin receptor cointernalized through clathrin-coated pits, coated vesicles, endosomes, and multivesicular bodies. Products of mutant c-erbB-2 that lacked a portion of the tyrosine kinase domain or that lacked most of the cytoplasmic domain were endocytosed in the presence of TA1 as promptly as the wild-type c-erbB-2 product. Slightly more rapid internalization of TA1 was observed in rat cells that expressed c-erbB-2 with a single point mutation in the transmembrane domain. Taken together, our data suggest that neither the intracytoplasmic domain nor receptor phosphorylation is required for antibody-mediated endocytosis of c-erbB-2.

9/7/28
DIALOG(R)File 155:MEDLINE(R)

07018181 91317780
p185HER2 signal transduction in breast cancer cells.
Scott GK; Dodson JM; Montgomery PA; Johnson RM; Sarup JC; Wong WL; Ullrich A; Shepard HM; Benz CC
Cancer Research Institute, University of California, San Francisco 94143.
J Biol Chem (UNITED STATES) Aug 5 1991, 266 (22) p14300-5, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: CA-44768, CA, NCI; CA-36773, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A partially agonistic monoclonal antibody, 4D5, known to bind to the extracellular domain of p185HER2 and shown to inhibit long term growth of p185HER2-overexpressing breast cancer cells, was used to study signal transduction and phosphotyrosyl protein substrates associated with this receptor. Normal breast epithelial cells and breast carcinoma cells expressing low levels of p185HER2 were not affected by 4D5. HER2/neu-overexpressing breast cancer cells (BT-474 and SK-Br-3) exposed to 4D5 exhibited rapid phosphorylation of both p185HER2 and an associated 56-kDa phosphotyrosyl protein (ptyr56). Paralleling the 4D5- stimulated phosphorylation of p185HER2 and ptyr56 was a 5-10-fold induction of c-fos mRNA and phosphatidylinositol 4-kinase activity and a 2-fold induction of inositol 1,4,5-trisphosphate 3'-kinase activity. The increased phosphatidylinositol 4-kinase activity immunoprecipitated with p185HER2 and also co-eluted with ptyr56 from an antiphosphotyrosine immunoaffinity column. These results indicate that short term (less than 6 h) 4D5 activation of p185HER2 in overexpressing breast cancer cells produces agonistic-like signaling typical of homologous tyrosine kinase growth factor receptors such as epidermal growth factor receptor. The data also suggest that ptyr56 represents a novel phosphorylated substrate associated with 4D5-stimulated p185HER2.

9/7/29
DIALOG(R)File 155:MEDLINE(R)

07014359 91215596
An antigen immunologically related to the external domain of gp185 is shed from nude mouse tumors overexpressing the c-erbB-2 (HER-2/neu) oncogene.
Langton BC; Crenshaw MC; Chao LA; Stuart SG; Akita RW; Jackson JE
Department of Immunology, Berlex Biosciences Inc., Alameda, California 94501.
Cancer Res (UNITED STATES) May 15 1991, 51 (10) p2593-8, ISSN 0008-5472 Journal Code: CNF
Languages: ENGLISH
Document type: JOURNAL ARTICLE
An antigen, immunologically related to the external domain of the c-erbB-2 (HER-2/neu) protein, was found shed into the serum of nude mice bearing tumors that overexpress the c-erbB-2 protein (gp185). Utilizing paired combinations from a panel of monoclonal antibodies (TAbs 250-265), with specificity for extracellular epitopes of gp185, an immunoradiometric assay was developed to quantitate this shed antigen. The immunoradiometric assay detected membrane-bound and soluble gp185 as well as a soluble derivative corresponding in sequence to the extracellular domain of gp185 (designated gp75). This recombinantly expressed gp75 was immunoaffinity purified and used to generate a standard curve from which serum samples were quantitated. Increases in antigen levels measured in the sera of tumor-bearing nude mice correlated with both overexpression of the c-erbB-2 protein and increased tumor volume. Positive sera were obtained from mice given implants of NIH3T3 cells transfected with c-erbB-2 complementary DNA (NIH3T3t), or ovarian (SK-OV-3) or breast (MDA-MB-361) tumor cell lines overexpressing the c-erbB-2 protein. In mice bearing NIH3T3t tumors, increases in tumor volume from 80 to 9000 mm3 resulted in levels of shed antigen from 8 to greater than 1000 ng/ml gp75 equivalents. Sera from mice with c-erbB-2-negative tumors or tumors overexpressing the epidermal growth factor receptor were negative in the assay. This assay, and the quantitation of shed antigen levels, may have diagnostic or monitoring utility in cancers, such as breast and ovarian, in which the c-erbB-2 protein is overexpressed.

9/7/30
DIALOG(R)File 155:MEDLINE(R)

07013431 91184915
Selection of monoclonal antibodies which induce internalization and phosphorylation of p185HER2 and growth inhibition of cells with HER2/NEU gene amplification.
Tagliabue E; Centis F; Campiglio M; Mastroianni A; Martignone S; Pellegrini R; Casalini P; Lanzi C; Menard S; Colnaghi MI
Division of Experimental Oncology E, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy.
Int J Cancer (UNITED STATES) Apr 1 1991, 47 (6) p933-7, ISSN 0020-7136 Journal Code: GQU
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In order to obtain further information on the biological role of the HER2/neu oncoprotein monoclonal antibodies (MAbs) were produced against the p185 extracellular domain. To immunize the mice and screen the hybridoma supernatants we selected a lung adenocarcinoma cell line (Calu-3), which demonstrated an over-expression of p185HER2 measured as the reactivity with

polyclonal rabbit serum to the 14-amino-acid carboxy-terminal-HER2/neu. Two MAbs, designated MGR2 (IgG1) and MGR3 (IgG2), selected for reactivity on Calu-3 and negativity on A43I live cells, the reference target cell for EGF receptor expression, were found to immunoprecipitate a 185-kDa molecule. Immunodepletion experiments with the polyclonal antiserum and cross-competition experiments indicated that the 2 reagents recognized 2 different epitopes located on the p185HER2 molecule. One of the 2 MAbs, MGR3, was found to internalize, induce p185HER2 phosphorylation and inhibit tumor cell growth in vitro. These results indicate that MGR3 is directed against a determinant located in the p185HER2 ligand binding site and may compete with the p185HER2 ligand, but is incapable of inducing a complete mitotic signal.

9/7/31
DIALOG(R)File 155:MEDLINE(R)

07011874 91107672
The extracellular domain of p185/neu is released from the surface of human breast carcinoma cells, SK-BR-3.
Zabrecky JR; Lam T; McKenzie SJ; Carney W
Applied bioTechnology, Inc., Cambridge, Massachusetts 02142.
J Biol Chem (UNITED STATES) Jan 25 1991, 266 (3) p1716-20, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The human breast carcinoma cell line SK-BR-3, expresses the neu oncogene product, p185, which is a receptor tyrosine kinase. Using a double monoclonal antibody capture enzyme-linked immunosorbent assay for p185, activity was detected in conditioned media from cultures of SK-BR-3 cells. Two monoclonal antibodies specific for the extracellular domain of p185/neu immunoprecipitated a protein with a molecular mass of approximately 105 kDa. p105 was further shown to compete with p185 for binding to monoclonal antibodies and pulse-chase experiments indicate that it was generated by post-translational processing. Peptide maps showed that p105 and p185 are related polypeptides. Since p105 is close to the predicted size for the extracellular domain of p185/neu, we propose that SK-BR-3 cells specifically process and release this portion of the receptor into the medium. The release of the extracellular domain may have implications in oncogenesis and its detection could prove useful as a cancer diagnostic.

9/7/32
DIALOG(R)File 155:MEDLINE(R)

06692535 91108899
Expression of c-erbB-2 gene product in urinary bladder cancer.
Moriyama M; Akiyama T; Yamamoto T; Kawamoto T; Kato T; Sato K; Watanuki T ; Hikage T; Katsuta N; Mori S
Department of Pathology and Oncology, Institute of Medical Science, University of Tokyo, Japan.
J Urol (UNITED STATES) Feb 1991, 145 (2) p423-7, ISSN 0022-5347 Journal Code: KC7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Expression of the c-erbB-2 gene product and the epidermal growth factor

receptor (EGF-R) was investigated in 54 cases of human bladder cancer immunohistologically and by Western blot analysis. For detection of the c-erbB-2 product, two specific antibodies, a rabbit polyclonal antibody directed to the intracellular domain and a murine monoclonal antibody recognizing an epitope in the extracellular domain, were used. Seventeen cases of bladder cancer were stained by the anti-c-erbB-2 polyclonal antibody, while 20 cases were stained by the monoclonal antibody, with good correlation on both stainings (p less than 0.01). There were four c-erbB-2 positive cases in 26 G1 tumors, four in 15 G2 tumors, and nine in 13 G3 tumors. There were also eight erbB-2 positive cases in nine muscle-invasive tumors, nine of 45 superficial tumors, four of five with lymph node metastasis, and seven of 14 without metastasis, as revealed by staining with the polyclonal antibody. Thus, the c-erbB-2 gene product was more frequently expressed in high grade tumors (p less than 0.01), in high stage tumors (p less than 0.01), and nodal metastatic tumors (N.S. by Chi-square test). Twenty-two of the 54 tumors were stained by an anti-EGF-R monoclonal antibody, 528 IgG. The expression of EGF-R was independent of histological grading, tumor stage, and nodal status, and no correlation was observed between expression of the c-erbB-2 product and EGF-R. The c-erbB-2 product may be applicable as a tumor marker for evaluation of malignant potential, invasiveness, and probably metastatic potential of human bladder cancer.

9/7/33
DIALOG(R)File 155:MEDLINE(R)

06687199 91329303
The normal erbB-2 product is an atypical receptor-like tyrosine kinase with constitutive activity in the absence of ligand.
Lonardo F; Di Marco E; King CR; Pierce JH; Segatto O; Aaronson SA; Di Fiore PP
Laboratory of Molecular and Cellular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
New Biol (UNITED STATES) Nov 1990, 2 (11) p992-1003, ISSN 1043-4674 Journal Code: AZH
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Overexpression of the erbB-2/neu gene is frequently detected in human cancers. When overexpressed in NIH 3T3 cells, the normal erbB-2 product, gp185erbB-2, displays potent transforming ability as well as constitutively elevated levels of tyrosine kinase activity in the absence of exogenously added ligand. To investigate the basis for its chronic activation we sought evidence of a ligand for gp185erbB-2 either in serum or produced by NIH 3T3 cells in an autocrine manner. We demonstrate that a putative ligand for gp185erbB-2 is not contained in serum. Chimeric molecules composed of the extracellular domain of gp185erbB-2 and the intracellular portion of the epidermal growth factor receptor (EGFR) did not show any transforming ability or constitutive autophosphorylation when they were expressed in NIH 3T3 cells. However, they were able to transduce a mitogenic signal when triggered by a monoclonal antibody directed against the extracellular domain of erbB-2. These results provide evidence against the idea that an erbB-2 ligand is produced by NIH 3T3 cells. Furthermore, we obtained direct evidence of the constitutive enzymative activity of gp185erbB-2 by demonstrating that the erbB-2 kinase remained active in a chimeric configuration with the extracellular domain of the EGFR, in the absence of any detectable ligand for the EGFR. Thus, under conditions of overexpression, the normal gp185erbB-2 is a constitutively active kinase

able to transform NIH 3T3 cells in the absence of ligand.

9/7/34
DIALOG(R)File 155:MEDLINE(R)

06685243 91145180
The presence of c-erbB-2 gene product-related protein in culture medium conditioned by breast cancer cell line SK-BR-3.
Alper O; Yamaguchi K; Hitomi J; Honda S; Matsushima T; Abe K
Growth Factor Division, National Cancer Center Research Institute, Tokyo, Japan.
Cell Growth Differ (UNITED STATES) Dec 1990, 1 (12) p591-9, ISSN 1044-9523 Journal Code: AYH
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The Mr 185,000 glycoprotein encoded by human c-erbB-2/neu/HER2 gene, termed c-erbB-2 gene product, shows a close structural similarity with epidermal growth factor receptor and is now regarded to be a growth factor receptor for an as yet unidentified ligand. Abundant c-erbB-2 mRNA was demonstrated by Northern blot studies in the human breast cancer cell line SK-BR-3. Cellular radiolabeling experiments followed by immunoprecipitation with three different anti-c-erbB-2 gene product antibodies, recognizing extracellular domain, kinase domain, and carboxyl-terminal portion, respectively, demonstrated the production of a large amount of c-erbB-2 gene product which had the capacity to be phosphorylated. Immunization of mice with concentrated culture medium conditioned by SK-BR-3 cells always generated antibodies against c-erbB-2 gene product, demonstrating that this culture medium contained substance(s) immunologically indistinguishable from c-erbB-2 gene product. This observation was supported by the successful development of a monoclonal antibody against c-erbB-2 gene product, GFD-OA-p185-1, by immunizing mice with this culture medium. The biochemical nature of the substance(s) present in the culture medium was further characterized. When the culture medium conditioned by [35S]cysteine-labeled SK-BR-3 cells was immunoprecipitated by three different anti-c-erbB-2 gene product antibodies, only the antibody recognizing extracellular domain precipitated the [35S]-labeled protein with a molecular weight of 110,000, namely p110. The newly developed monoclonal antibody also immunoprecipitated this protein.(ABSTRACT TRUNCATED AT 250 WORDS)

9/7/35
DIALOG(R)File 155:MEDLINE(R)

06684706 91119659
Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen.
Bacus SS; Kiguchi K; Chin D; King CR; Huberman E
Cell Analysis Systems, Inc., Elmhurst, Illinois.
Mol Carcinog (UNITED STATES) 1990, 3 (6) p350-62, ISSN 0899-1987 Journal Code: AEQ
Contract/Grant No.: IR43CA50843-01, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The relationship between terminal cell differentiation and changes in the

subcellular levels of the HER-2/neu antigen was investigated in cultured human breast cancer cells: AU-565 cells (which overexpress the HER-2/neu gene) and MCF-7 cells (which do not overexpress this gene). Differentiation was achieved by treating the cells with mycophenolic acid (MPA), phorbol 12-myristate 13-acetate (PMA), retinoic acid (RA), or the TA-1 monoclonal antibody to the extracellular domain of the HER-2/neu protein. Ten to twenty percent of the cells in untreated, sparsely growing AU-565 cultures exhibited morphological maturation characterized by large lacy nuclei surrounded by sizable flat cytoplasms. A fraction of these cells harbored milk factors such as casein and large lipid droplets. Treatment of the AU-565 cells for 4 d with 9 microM MPA, 1.6 nM PMA, 2.5 microM RA, or 1 microgram/mL TA-1 antibody resulted in cell growth inhibition and an increase in the percentage of cells (48-97%) that exhibit a mature phenotype. MCF-7 cells were also susceptible to differentiation by MPA and RA, but to a lesser degree than the AU-565 cells. Differentiation in the AU-565 and MCF-7 cells was associated with reduced immunostaining for the HER-2/neu protein at the cell surface membrane and with a transient increased diffuse immunostaining for this protein in the cytoplasm.

9/7/36
DIALOG(R)File 155:MEDLINE(R)

06679608 90361943
ELISA for quantitation of the extracellular domain of p185HER2 in biological fluids.
Sias PE; Kotts CE; Vetterlein D; Shepard M; Wong WL
Department of Immunology Research and Assay Technologies, Genentech Inc., So. San Francisco, CA 94080.
J Immunol Methods (NETHERLANDS) Aug 28 1990, 132 (1) p73-80, ISSN 0022-1759 Journal Code: IFE
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The HER2/neu proto-oncogene encodes a receptor that belong to the tyrosine-specific protein kinase family. Amplification of the HER2 gene in patients with breast and ovarian cancer has been shown to predict poorer survival rates. In order to understand the role of HER2 in malignant and normal cells, it is necessary to devise assays that can quantitate expression levels of the HER2 gene product (p185HER2) in production samples, biopsy specimens and biological fluids. We have developed a simple, quantitative ELISA that uses two monoclonal antibodies directed against the extracellular domain of the HER2 gene product, p185HER2 (HER2 ECD). The assay has a detection range of 0.25-120 ng/ml, is precise and sensitive. The ability of this assay to detect biologically active rHER2 ECD is demonstrated by its correlation to a growth inhibitory bioassay (r = 0.92). The sandwich ELISA can also accurately quantitate rHER2 ECD in mouse and monkey serum. This assay should be useful for quantitating low levels of circulating rHER2 ECD in animals in which rHER2 ECD is being used as antigen for immunotherapy and in patients which 'shed' receptor.

9/7/37
DIALOG(R)File 155:MEDLINE(R)

06676811 90283901
Overexpression of HER-2/neu is associated with poor survival in advanced

epithelial ovarian cancer.
Berchuck A; Kamel A; Whitaker R; Kerns B; Olt G; Kinney R; Soper JT; Dodge R; Clarke-Pearson DL; Marks P; et al
Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, North Carolina 27710.
Cancer Res (UNITED STATES) Jul 1 1990, 50 (13) p4087-91, ISSN 0008-5472 Journal Code: CNF
Contract/Grant No.: 5R01-CA39930, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Previous studies have suggested that overexpression of HER-2/neu oncogene occurs in 15-40% of breast cancers and that overexpression is associated with poor prognosis. In the present report, we have used an immunohistochemical technique involving a monoclonal antibody specifically reactive with the external domain of HER-2/neu to study expression of HER-2/neu in frozen sections of normal ovary and advanced epithelial ovarian cancer. The intensity of staining for HER-2neu was always moderate or less (0-2+) in normal ovarian epithelium. Among 73 ovarian cancers, 50 (68%) had staining similar to that for normal ovarian epithelium (0-2+) while 23 (32%) stained heavily (3+). Survival of the 23 patients with high HER-2/neu expression (median, 15.7 months) was significantly worse (P = 0.001) than that of the 50 patients (median, 32.8 months) with normal HER-2/neu expression. In addition, patients whose tumors had high HER-2/neu expression were significantly less likely to have a complete response to primary therapy (P less than 0.05) or have a negative second-look laparotomy when serum CA 125 levels were normal preoperatively (P less than 0.05). These findings suggest that HER-2/neu deserves further evaluation as a prognostic marker in epithelial ovarian cancer.

9/7/38
DIALOG(R)File 155:MEDLINE(R)

06673027 90170193
Expression of the p185 encoded by HER2 oncogene in normal and transformed human tissues.
Natali PG; Nicotra MR; Bigotti A; Venturo I; Slamon DJ; Fendly BM; Ullrich A
Regina Elena Cancer Inst., Rome, Italy.
Int J Cancer (UNITED STATES) Mar 15 1990, 45 (3) p457-61, ISSN 0020-7136 Journal Code: GQU
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The human homolog of the rat neu oncogene, HER2 (also termed c-erbB2) has been demonstrated in amplified form in human breast tumors with poor prognosis. Although amplification of the gene correlates with expression of a 185-kDa transmembrane glycoprotein, no extensive information is available regarding the extent of tissue and tumor specificity of this gene product. We have addressed this issue by immunohistochemically evaluating the expression of p185 HER2 in normal tissue and various tumors using monoclonal antibodies (MAbs) to distinct epitopes of its extracellular domain. No detectable levels of p185 HER2 were found in fetal tissues analyzed, with the exception of renal tubules in 2 out of 3 specimens tested and in intestinal epithelium. In adult tissues, detectable levels of this glycoprotein were found in a restricted number of cell types, the expression being heterogeneous among individuals and cell histotypes. Among the neoplasms assayed p185 HER2 was expressed in 46% of primary breast

cancers, in 28% of ovarian tumors and in 30% of colon rectum malignancies. No male breast adenocarcinomas were p185-positive. A large number of other tumors tested revealed only a low incidence of expression of the p185. In metastatic breast tumors p185 HER2 was demonstrated homogeneously among multiple autologous lesions and almost invariably (80%) the expression of p185 in the primary lesion correlated with that of the deriving metastases. Our findings indicate that the expression of the p185 HER2 represents a tumor marker of clinical relevance in breast cancer. Whether this holds true for other malignancies remains to be explored.

9/7/39
DIALOG(R)File 155:MEDLINE(R)

05914011 90058585
Analysis of c-erbB-2 expression in breast carcinomas with clinical follow-up.
Thor AD; Schwartz LH; Koerner FC; Edgerton SM; Skates SJ; Yin S; McKenzie SJ; Panicali DL; Marks PJ; Fingert HJ; et al
Department of Pathology, Massachusetts General Hospital, Boston 02114.
Cancer Res (UNITED STATES) Dec 15 1989, 49 (24 Pt 1) p7147-52, ISSN 0008-5472 Journal Code: CNF
Contract/Grant No.: P01 CA44768, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Various monoclonal antibodies reactive with protooncogene products or tumor-associated antigens have been utilized to investigate breast carcinoma biology or antigen expression with potential prognostic relevance. Murine monoclonal antibody TA1, generated by immunization of BALB/c mice with whole c-erbB-2 (neu) transformed NIH/3T3 cells, recognizes the extracellular domain of the c-erbB-2 protein and binds a Mr 185,000 protein by immunoprecipitation. Using avidin-biotin-peroxidase techniques and monoclonal antibody TA1, 313 archival primary adenocarcinomas of the breast were evaluated for c-erbB-2 overexpression; 290 of these were used for multiparametric statistical analysis. Historical, clinical (age, laterality), histological (nuclear grade, tumor size, lymph node status, lymphatic or blood invasion), and hormone receptor data as well as clinical outcome (minimal follow-up, 6 years; median follow-up, 8.5 years) were compared to TA1 staining. For these 290 patients Cox regression multivariate analysis showed the strongest correlation between lymph node status or estrogen receptor status and overall survival (P = 0.0001 and 0.049, respectively). TA1 staining did not significantly correlate with survival (P = 0.395). However, univariate analysis of certain patient subpopulations showed a significant correlation if the examined tumors were subdivided into negative or focally reactive and those with greater than or equal to 40% cellular reactivity. For T3, T4 patients, strong TA1 immunoreactivity correlated with a shortened disease-free survival (log rank P = 0.0018; Wilcoxon p = 0.0078) and overall survival (log rank P = 0.0002; Wilcoxon P = 0.0013). For these patients the overall survival at 6 years was markedly different between the strongly reactive tumors (0%) and the negative to weakly reactive tumors (55%). In lymph node-positive patients a trend between high TA1 reactivity and a worse overall survival was also noted (log rank P = 0.128; Wilcoxon P = 0.054), with a 6-year survival of 42% in the strongly reactive tumors (n = 16) and 65% in the negative to weakly reactive carcinomas (n = 105). No correlation between TA1 immunoreactivity and other historical, clinical, and histological features were noted. c-erbB-2 overexpression as measured by

immunohistochemical techniques, therefore, may have clinical significance in certain patient subpopulations.

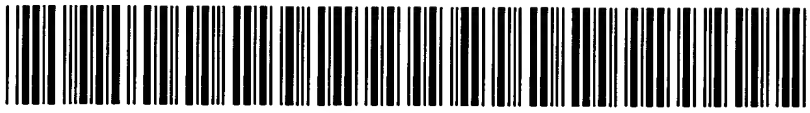
9/7/40
DIALOG(R)File 155:MEDLINE(R)

05907781 89263246
Generation and characterization of monoclonal antibodies specific for the human neu oncogene product, p185.
McKenzie SJ; Marks PJ; Lam T; Morgan J; Panicali DL; Trimpe KL; Carney WP
Applied bioTechnology, Cambridge, Massachusetts 02142.
Oncogene (ENGLAND) May 1989, 4 (5) p543-8, ISSN 0950-9232
Journal Code: ONC
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A series of monoclonal antibodies specific for the extracellular domain of the human neu gene product (p185) have been produced. The generation of these monoclonal antibodies, and their biochemical and immunological characterization is described. The immunization protocol utilized a series of injections of NIH3T3 cells, cyclophosphamide, and a neu transfected NIH3T3 cell line (designated 18-3-7) which expressed the full length human neu-encoded protein. This immunization regimen induced an immune response to the extracellular portion of p185 on the 18-3-7 cells. A panel of ten hybridomas were identified which secreted monoclonal antibodies with a variety of epitope specificities, and reacted with p185 in a number of different experimental formats. As the neu gene product has been associated with human breast cancers, a series of monoclonal antibodies such as these could prove useful in the diagnosis, prognosis and/or treatment of these human malignancies.

9/7/41
DIALOG(R)File 155:MEDLINE(R)

05907732 89261720
p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor.
Hudziak RM; Lewis GD; Winget M; Fendly BM; Shepard HM; Ullrich A
Department of Developmental Biology, Genentech, Inc., South San Francisco, California 94080.
Mol Cell Biol (UNITED STATES) Mar 1989, 9 (3) p1165-72, ISSN 0270-7306 Journal Code: NGY
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The HER2/c-erbB-2 gene encodes the epidermal growth factor receptorlike human homolog of the rat neu oncogene. Amplification of this gene in primary breast carcinomas has been show to correlate with poor clinical prognosis for certain cancer patients. We show here that a monoclonal antibody directed against the extracellular domain of p185HER2 specifically inhibits the growth of breast tumor-derived cell lines overexpressing the HER2/c-erbB-2 gene product and prevents HER2/c-erbB-2-transformed NIH 3T3 cells from forming colonies in soft agar. Furthermore, resistance to the cytotoxic effect of tumor necrosis factor alpha, which has been shown to be a consequence of HER2/c-erbB-2 overexpression, is significantly reduced in the presence of this antibody.

US 084881800AP1



Creation date: 10-17-2003
Indexing Officer: NNGUYEN7 - NAM NGUYEN
Team: OIPEBackFileIndexing
Dossier: 08488180

Legal Date: 10-11-2001

No.	Doccode	Number of pages
1	CTNF	11
2	892	1
3	NPL	5
4	NPL	19

Total number of pages: 36

Remarks:

Order of re-scan issued on .....